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Investigation of Blood Lipid Changes in Horses Infected With Equine Infectious Anemia.

Donald Gene Luther
Louisiana State University and Agricultural & Mechanical College

INVESTIGATION OF BLOOD LIPID CHANGES IN HORSES INFECTED WITH EQUINE INFECTIOUS ANEMIA

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TO

TAMA, MARTY, BINKY, TENINCY, AND ?

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ABSTRACT

The fatty acids of plasma and erythrocytes from horses were analyzed before and after infection with equine infectious anemia virus (EIA). Changes in the composition of fatty acids were followed by gas-liquid chromatographic (GLC) analysis of the total lipid extract and the various lipid classes of both the plasma and erythrocytes.

The principle fatty acids detected in the plasma and erythrocytes were C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C22:0, and C24:0. Erythrocytes contained a higher percentage of long chain fatty acids than the plasma. The erythrocytes also contained C24 fatty acids of undetermined saturation which increased in concentration as EIA progressed. Lipid class analyses showed that these C24 fatty acids were present in the phospholipid (PL) and sterol ester (SE) fractions.

The erythrocytes had a ratio of saturated to unsaturated fatty acids usually greater than 1, but in acute cases of EIA it decreased to 0.38. This decrease in the ratio was due to a marked increase in C24 unsaturated fatty acids.

The triglyceride (TG) fraction of the plasma which was observed on thin-layer chromatographic (TLC) plates under ultra-violet light exhibited progressive increases in concentration in the plasma throughout the course of EIA.

The SE fraction of the plasma had the lowest ratio of saturated to unsaturated fatty acids. The ratio varied between 0.22 and 0.13 throughout the course of experimental EIA. The concentration of C18:2 ranged from 73.73 percent to 53.23 percent during the course of EIA. The concentration of the SE fraction decreased as determined on TLC plates as the disease progressed. In the preinoculation period the total lipid extract of the plasma contained approximately 40 percent of C18:2 which decreased through the course of the disease while the concentration of C18:1 increased.

INTRODUCTION

Equine infectious anemia (EIA) has been recognized as a serious infectious disease of horses for almost 130 years (Dreguss and Lombard, 1954). The causative agent of the disease is a virus and is found in all tissues and fluids of the infected horse. Biting insects are the natural vectors of EIA; however, the most recent outbreaks of EIA have been attributed to the use of contaminated syringes and needles on horses.

The inability of researchers to find a suitable host other than the equine species presented numerous problems to investigators prior to the successful attempt by Kobayashi and Kono (1967) to propagate EIA virus in horse leukocyte cultures.

EIA is manifested in three forms: acute, subacute, and chronic. A hemolytic anemia is produced by EIA virus and there is a marked lipemia which develops as the disease progresses.

This investigation was initiated to study the possibility that lipids were being produced during the course of the disease which could be responsible for the hemolytic anemia of EIA. In this investigation the total lipids of plasma and erythrocytes were extracted and analyzed by GLC. The lipids of the plasma and erythrocytes were separated into

classes (PL, free fatty acid (FFA), TG, and SE) by TLC and analyzed by GLC. The changes in the fatty acids before inoculation and during the course of the disease are reported in this dissertation.

REVIEW OF LITERATURE

A. Early History of EIA

Equine infectious anemia is a viral disease of the equine species, and is mainly characterized by intermittent fever, marked depression, progressive weakness, loss of weight, marked ventral edema, congestion, icterus and anemia (Dreguss and Lombard, 1954).

First reported in Europe in 1843, EIA is considered today as a serious disease with a world-wide distribution. The first recognized cases of EIA in North America occurred in Canada about 1880. The disease has existed for about 75 years in the United States. Natural outbreaks of EIA have been confined to relatively small areas and it has shown little tendency to spread. However, with modern transportation of animals, a potential danger of epizootics exists (Knowles, 1969).

The natural transmitting vectors of EIA are biting insects, but use of contaminated hypodermic syringes and needles is the most common method of transmitting EIA today.

Natural outbreaks of EIA are more prevalent in poorly-drained, low-lying sections, but it has also been found at high elevations on well-drained pastures. The incidence of the disease increases in the warm months of the

year when biting insects are more abundant (Dreguss and Lombard, 1954).

Under natural conditions the virus appears to affect only the equine species. It may persist for life in a carrier state in animals who have recovered from the disease. The virus shows considerable resistance to disinfectants, heat, freezing, and drying (Stein and Mott, 1947).

The clinical disease syndrome manifests itself in three forms: acute, subacute and chronic. The acute form is characterized by a sudden, rapid rise in body temperature which may reach 108° F. The temperature may either fluctuate or remain elevated until death. The more common, chronic form of EIA is characterized by intermittent attacks of fever, loss of weight, progressive weakness, marked depression and edema in the lower extremities (Dreguss and Lombard, 1954). Following the intermittent febrile attacks, the disease is generally progressive. Asymptomatic periods are often interrupted by prolonged periods of clinical symptoms of EIA. A persistent viremia occurs in both asymptomatic carrier and during the active case of EIA.

B. Pathological Changes in EIA

1. Macroscopic Changes in Equine Species.

The macroscopic changes associated with EIA vary with the form of the disease. In acute and subacute cases, severe hemorrhages are visable on the serous and mucous membranes of the liver, heart, kidney and intestines. The intestines are

sometimes filled with blood from hemorrhages of the intestinal mucosa. The liver is frequently enlarged as is the heart, kidney and spleen. The spleen is purple-red in color and has black homorrhagic foci scattered over the surface. It is swollen, congested, engorged with blood and the cut parenchyma tissue appears granular. Visceral lymph nodes are swollen and red due to hyperemia and hemorrhage. The liver appears cloudy and has an icteric appearance with numerous hemorrhagic foci. Kidneys are swollen, anemic and icteric (Knowles, 1969).

In the chronic form of EIA, the hyperemia and edema seen in the acute disease are reduced in the kidney, liver, spleen and lymph nodes. There is a proliferation of argyrophilic fibers. These organs undergo some atrophy. Splenic atrophy results in a thickened capsule with an elastic parenchyma. Splenic pulp becomes reddish and follicles are grossly recognizable. The trabeculae are very distinct on cut surfaces. The liver becomes atrophied in chronic cases and appears pale as the anemia becomes more pronounced. The kidney and lymph nodes undergo atrophy and become quite pale as time elapses. Active sites of hemorrhage are usually absent in chronic forms of EIA (Dreguss and Lombard, 1954).

2. Histological Changes in EIA.

The histopathological changes caused by the EIA virus varies with the form of disease syndrome; i.e., acute, subacute and chronic. Ishitani (1966) found characteristic histological lesions in the spleen, liver, lymph nodes, kidney, heart and

lungs in 120 EIA-infected horses. The primary lesions in the spleen in acute and subacute cases were marked degeneration and decreased numbers of lymph follicles, severe edema and engorgement with debris from destroyed red blood cells. The red pulp was hemorrhagic and congested with focal aggregation of basophilic round cells around the central and sheathed arteries. Yamamoto and Kono (1967) reported that the fine structure of the basophilic round cells closely resemble the plasma cells or their precursors.

In acute and subacute cases the visceral lymph nodes exhibited karyopyknotic and karyorrhectic degeneration of lymphocytes. The central core of the lymph follicles exhibited marked atrophy.

The kidney, lungs and adrenal glands in acute and subacute cases of EIA exhibit edema, cellular degeneration, hemorrhage and increased infiltration of histocytic cells. The bone marrow showed massive hemorrhage in the acute and subacute forms of the disease. Yamamoto and Kono (1967) did not consider hemorrhage to be the chief pathological change in the bone marrow, but believed that the change in the density and extent of the bone marrow was the main area of pathology.

In chronic cases of EIA the major lesions are found in the liver. The histopathological changes are characterized by small round cell infiltration along the sinusoids and slight activation of the Kupffer and endothelial cells of the sinusoids. Yamamoto and Kono (1967) also observed multiple formations of

small nodules in the liver parenchyma with infiltration of lymphoid cells in Glisson's sheaths. The spleen in chronic cases showed an increase in size and number of lymphoic follicles, with a rich infiltration of lymphoic cells around the central artery. The visceral lymph nodes had enlarged and increased numbers of lymph follicles. There was only a slight increase in cellular bone marrow density in chronic cases.

C. Characteristics of EIA Virus

1. In Vivo Distribution of EIA Virus.

EIA virus can be isolated from a recovered animal for life. Stein et al. (1955) reported a recovered case from which he was able to transmit the disease 18 years later through blood transfer. During all stages of active EIA, the bone marrow, spleen, liver and visceral lymph nodes contain high concentrations of the virus. It has also been isolated from feces, urine, tears and semen from infected horses.

2. Morphology of EIA virus.

In 1939 Balozet with the aid of filters estimated that EIA virus had a particle size of 18 to 50 mu in diameter. Using the electron microscope to visualize the virus Reagan et al. (1950) described a spherical particle in the sediment of serum from an EIA-infected horse to have a size range of 11 to 49 mu. Ishii et al. (1953) in an electron microscope study found a particle with a size of 20 to 50 mu in the serum of infected horses.

Moore (1969), attempted isolation of EIA virus in a pure form from horse leukocyte cultures and recovered several fractions which adsorbed in the range of 260 - 280 mu. Moore was interested in the nature of EIA virus and its nucleic acid content. Fraction I, which was infectious, contained DNA. A particle in the range of 30 to 50 mu was consistently demonstrated with the electron microscope. This particle was extremely sensitive to ether as is EIA virus.

Boyd (1971), in her attempts to isolate and characterize EIA virus, was able to demonstrate with an electron microscope two distinct particles sizes. The virons were spherical to pleomorphic and measured 45 - 50 mu and 70 - 120 mu in diameter. DNA was found in the purified virions. A horse inoculated with these purified particles developed clinical symptoms of EIA.

3. Growth of EIA Virus.

The search for a host other than the equine species in which to grow EIA virus has included all of the usual laboratory animals, as well as sheep, goats and pigs; however, an alternate host has not been found. Saurino et al. (1957) attempted to grow the virus in embryonated chicken eggs. They were unable to demonstrate the presence of the virus in a second passage to embryonated eggs when inoculations were made into a susceptible animal.

The use of tissue cultures suggested another method to researchers who wished to grow the virus. Kobayashi and Kono (1967) reported success in growing the virus in tissue

cultures of horse leukocytes. EIA virus produced a type of cytopathic effect (CPE) in horse leukocytes, but workers could not be sure that the effect was actually due to the virus as complement fixing (CF) and virus neutralization techniques had not yet been proven accurate.

Kono and Kobayashi (1967) attempted to prove that the CPE and CF antigen exhibited in horse leukocyte cultures were specific for EIA virus. The presence or absence of transmissibility of CPE was checked by individual serial passages in horse leukocyte cultures. Clinical cases of EIA developed when CPE-positive cultures were inoculated into susceptible horses, but no response was noted when cultures with no CPE were inoculated. The CPE was concluded to be a specific indication for the propagation of the EIA virus.

Kono et al. (1970) reported that the CPE could not be detected until 48 hours post-inoculation. Viral propagation was inhibited by adding 5-iodo-deoxyuridine. This inhibited DNA replication in the culture medium, which suggests that DNA synthesis is required for viral propagation.

The propagation of EIA virus in primary leukocytes provides many advantages over growth in the equine species. However, leukocyte cultures can only be maintained for about three weeks. Moore et al. (1970) reported improved methods of continuous passage of horse leukocyte cultures which gave the researcher extended time periods to observe the propagation of the virus and to demonstrate CPE.

Kono and Kobayashi (1970) discovered that the serial passage of EIA virus in horse leukocyte cultures brought about a change in the pathogenesis of the virus. The thirty-first passage did not cause clinical symptoms when it was inoculated into a susceptible horse; however, an early viremia was exhibited. The EIA disease response was not shown when the sixtieth passage was inoculated into a horse.

Various attempts have been made to purify EIA virus. Nakajima et al. (1967, 1968) reported growing the virus on horse leukocyte cultures. By employing ultracentrifugation, DEAE cellulose chromatography, and sucrose density gradient centrifugation, the virus was purified. Unfortunately, during the purification process, it became innocuous.

4. Hematology

Several research workers (Pehl, 1952; Dreguss and Lombard, 1954; Obara and Nakajima, 1961; McGuire et al., 1969a) have speculated on the pathogenesis of anemia in EIA. They propose that anemia is due to hypoplasia of the bone marrow and increased destruction of the red blood cells.

Carbrey (1969) stated that the hyperplasia of the bone marrow is attributed to the selective multiplication of lymphoid cells and not to increased compensating erythropoietic activity. He postulated that the reduced production of red blood cells is brought about by the destructive effect of population pressures from the proliferating lymphoid cells. Carbrey considered the anemia to be similar to leukemia, in which the bone marrow is infiltrated with leukemic cells

causing an aplastic anemia. An increased hemolysis as indicated by increased plasma hemoglobin was observed.

Matthias and Schmidt (1956) described inclusion bodies in the red blood cells of horses with EIA. These were similar to Heinz bodies which are associated with several anemias in man. Rothberg et al. (1959) demonstrated that erythrocytes containing Heinz bodies have a decreased half-life. McGuire et al. (1970) demonstrated that inclusion bodies present in the red blood cells of horses with EIA had a maximum range of 7 to 42 Heinz bodies per 1,000 cells. Normal horses have a range of 0 - 4 Heinz bodies per 1,000 red blood cells.

McGuire et al. (1970) indicated that although the Heinz bodies were present in larger numbers in horses with EIA, they were not significantly involved in the destruction of red blood cells.

Kaneko et al. (1969) suggested that enzymatic changes in the red blood cell membrane predisposed the cells to Heinz body formation, and that these enzymatic changes in the membrane of the red blood cells were the cause of hemolysis in EIA. McGuire et al. (1969) stated that the hemolysis was caused by antigen-antibody reaction on the surface of the red blood cells fixing complement with subsequent hemolysis. Even in the severe anemic conditions brought on by EIA reticulocytes and normoblasts were not observed in circulating blood, The equine species differ from man and several other animals in the absence of reticulocytes and other immature red blood cell precursors in the peripheral circulation in

response to loss or destruction of red blood cells. The mean corpuscular volume and mean corpuscular hemoglobin in horses with EIA increases slightly. This increase may be due to an early release of the larger and younger postreticulocyte red blood cells from the bone marrow.

The influx of basophilic round cells (large vacuolated mononuclear cells characteristic of horses with EIA) into the circulation causes an apparent monocytosis; however, the total white blood cell count decreases during the febrile periods of horses with EIA.

D. Immunological Response

Stein and Gates (1959) in an early report on the immune response, stated that serum from a horse which had naturally cleared itself of EIA virus would protect susceptible horses when virulent virus was experimentally inoculated. No other such report exists in the literature.

Kono (1969) reported a variation in production of CF antibody and neutralizing antibody in experimentally EIA-infected animals. Most of the horses exhibited some degree of CF antibody after the first fever peak, but horses that died in the peracute cases did not. Twenty-five horses were checked for the presence of neutralizing antibodies. Fourteen horses (56 percent) exhibited neutralizing antibody in the serum 120 days after the first fever spike, while 11 (44 percent) never exhibited neutralizing antibody. An explanation was not given why neutralizing antibody took 120 days to appear.

Kono (1968) stated that the CF antigen of EIA virus was not a surface antigen but was soluble. Kono's work indicated that this soluble CF antigen was actually an internal part of the virus particle.

Ushimi et al. (1969) commented on the ability of EIA-infected horses to react to an antigenic stimulus. Sheep red blood cells were used as the antigen and were injected into horses demonstrating acute and chronic disease. There was a tremendous increase in the antibody-producing cells in the early acute stage of EIA, with a corresponding increase in antibody. When injected into chronic cases of EIA, fewer cells were committed to the production of antibody. In the early stages of acute EIA, a tremendous proliferation and infiltration of the spleen and lymph nodes with basophilic round cells occurred. This might be the source of the increased production of antibody since the fine cellular structure of these cells closely resembled that of plasma cells.

E. Diagnosis of EIA

The lack of a valid test for the detection of EIA has caused problems in the attempts to control and eradicate the disease. Kono and Kobayashi (1966) reported a tissue culture antigen that exhibited CF properties but it was abandoned due to lack of specificity and inconsistent results. Dreguss and Lombard (1954) described the hemagglutination test for diagnosis of EIA but it lacked specificity and was discarded. Moore et al. (1966) described a precipitin test for detection of horses with EIA, but the test apparently detected the

circulation of an abnormal protein and not a specific EIA antibody. Saurino et al. (1966) described an immunoadherence test and a hemagglutination inhibition test, but they were not adopted because antibody could not be detected in the serum.

The most promising test today is the immunodiffusion test proposed by Coggins and Norcross (1970) in which a crude extract of the spleen from infected horses is utilized as the antigen. The test detects the presence of a circulating antibody in the serum of EIA-infected horses as early as 18 days post-inoculation. This antibody is believed to be specific for EIA virus. While the immunodiffusion shows promise, the only method currently accepted is animal inoculation and the appearance of clinical symptons of EIA.

F. Other Anemias

Anemias develop only when the rate of destruction of red blood cells exceeds the capacity of the bone marrow to produce erythrocytes. There are several other types of anemias whose pathological changes closely resemble those in the EIA disease syndrome. Other closely related anemias must be reviewed in order to eliminate the possibility that the pathological changes develop in the same target tissue as those of EIA.

1. Artificially-Induced

There are two major hypotheses about drug-induced immune hemolytic anemias. The first hypothesis is that the

drug forms a complex with a soluble blood macromolecule that acts as an antigen and which invokes an immune response by production of antibody. If the animal is reexposed to the drug, the antigen-antibody complex passively adsorbs to the surface of the red blood cell. The second hypothesis speculates that the red blood cell acts as a carrier protein for the drug antigen thus invoking an immune response. Reexposure produces identical results as in the first hypothesis (Shulman, 1964).

Cortisone-induced anemia and hyperlipemia are actually dilution anemias which result from increased lipids in the blood. There appears to be no relation to the hemolytic anemias brought on by cholesterol feeding and there is no change in the concentration of cholesterol or phospholipid in the red blood cells (Moran et al.,1966).

A number of drug-induced hemolytic anemias are associated with impairment of red blood cell metabolism. The incubation of red blood cells deficient in glucose-6-phosphate dehydrogenase (G-6-PD) with oxidizing drugs such as acetyl-phenyl-hydrazine causes loss of glutathione and formation of Heinz bodies. Denatured hemoglobin, resulting from hydrogen peroxide produced in the cell, attaches to the cell membrane, causing early destruction of the cell by the reticulo-endothelial system. Normal G-6-PD cells have the ability to produce sufficient TPNH to reduce the glutathione after exposure to hydrogen peroxide and thus prevent damage to the hemoglobin (Zalusky, 1970 and Jaffe, 1970b).

2. Hereditary Predisposition to Anemias.

Fourteen types of hereditary anemia caused by enzyme deficiencies were reviewed by Jaffe (1970a). Six of these enzyme deficiencies have not been well defined, with all cases relating to glycolytic enzymes. Mills et al. (1968) reported that red blood cells with low levels of ATP lost their biconcave shape and were predisposed to hemolysis by the reticulo-endothelial system. The low levels of ATP were believed to result from over-utilization of adenine nucleotide, which could have been causes by excess requirements of ATP for active transport across an altered cell membrane, excessive requirements for biosynthetic processes and/or loss due to excessive phosphatase activity inside the cell.

Unstable hemoglobin such as hemoglobin H, an inherited alteration in the Beta chain of the hemoglobin tetramer, will undergo hemolysis when exposed to sulfonamides both in vivo and in vitro (Rigas and Koler, 1961).

3. Lipid-Involved Hemolytic Anemias That Resemble EIA.

Okey and Greaves (1939) fed guinea pigs (GP) high levels of cholesterol. After five weeks on this diet, the GP became severely anemic and the liver developed enormous fatty infiltrations. These workers postulated that the liver lost its ability to esterify and hold back the extra cholesterol which exerted a surface tension effect on the red blood cell membrane resulting in hemolysis. Silver et al. (1964) fed rabbits high cholesterol diets which caused hyperlipemia, liver damage, enlarged spleens and severe anemia. Splenec-

tomies relieved the anemia only temporarily because the spleen is the major reticulo-endothelial organ for removal of damaged red blood cells. Silver et al. stated that the severity of the hemolytic anemia could be corrolated directly to the liver damage.

Westerman et al. (1970) reported on a biphasic anemia produced by cholesterol feeding. The first phase is a dilutional anemia caused by increased lipids in the blood. The second phase occurs when the ratio of plasma to red blood cell cholesterol is increased. As the red blood cholesterol increased the life span of the red blood cell decreased. The alteration in this relationship may be associated with decreased affinity of serum lipoprotein for free cholesterol. Hemolytic anemia is due to transfer of free cholesterol to the red blood cell or to the severity of associated liver damage. It is not due to free or esterified cholesterol in the serum.

Patients with Zieve's syndrome, i.e., hyperlipemia, which is caused by alcoholism and liver damage, exhibit hemolytic anemias. Westerman et al. (1968) postulated that these hemolytic anemias were caused by lipids coating the red blood cell and altering the cell membrane, thus shortening the life span.

Blass and Dean (1966) suggested that hyperlipemia and hemolytic anemia are independent of each other. Lysolecithin, a strong hemolytic agent, when more than doubled in concentration <u>in vivo</u> did not cause hemolysis. Hemolysis was believed to be due to liver damage, but they did not speculate on the mode of action.

Neerhout (1968) reported that patients with liver disease showed increased total lipid, lipid phosphorus and cholesterol. The most striking change in the erythrocyte stromal lipids was in the phospholipid fraction. Phosphatidyl choline increased two-fold, palmitic and oleic acids increased, but stearic and arachidonic acids decreased in concentration. The concentration of linoleic acid was variable. The red blood cell stromal lipid alterations could be attributed to increases in cholesterol and phosphatidyl choline. Neerhout believed that the red blood cell changes were associated with hepatic disease and should not be considered to be produced by nonspecific plasma hyperlipemia. Neerhout's hypothesis could be supported by the hyperlipemia caused by fasting (Zawilaki and Paluszak, 1970) and by stress in which hemolytic anemia was not present (Taggart and Carruthers, 1971).

Horses infected with EIA exhibited an increasingly altered lipoproteinemia as the disease syndrome became more pronounced. Horses usually had a grossly lipemic serum prior to death (Gainer et al., 1966a). Gainer et al. employed paper electrophoresis to measure the percentages of alpha lipoprotein (PAL) and beta lipoprotein (BL). The normal values for PAL were established at 56.9 percent. The animals were then experimentally infected with equine serum containing virulent EIA virus.

The PAL increased for 3 to 5 days post-inoculation, while the BL decreased. During the next 27 days the PAL

declined to a level of only 2 percent while the BL increased at least ten-fold. These workers pointed out that in a case of toxic hepatitis in a horse, the PAL dropped to zero (0) while the BL showed a great increase. When horses with equine piroplasmosis were tested for alterations in the PAL, the values were found to be in the normal range.

Gainer et al. made two principle observations: 1. As the temperature became elevated (biphasic temperature response), the lipoprotein values changed quite rapidly in a triphasic response. 2. The experimentally-infected animals did not experience a large decrease in packed red blood cell volume.

In a natural case of EIA, a decreased packed red blood cell volume that followed the altered PAL values was observed. The cause of the altered PAL were unknown but Gainer proposed several possibilities, including histopathological liver damage, direct extrusion from the fat deposits of the body, destruction of erythrocytes with lipid release and low levels of lipoprotein lipase in EIA-infected horses.

Gainer et al. suggested that the target tissue for the EIA virus was the hepatic cord cells. The liver function was then altered to such an extent that it could no longer properly metabolize serum lipids, which resulted in an increased BL.

The normal horse has a serum lactic dehydrogenase (LDH) range of 200 to 830 Berger-Broida units (BBU) with a mean value of 572 BBU. Gainer et al. (1966b) found elevations up to 19,600 BBU in the serum of a horse terminal with EIA.

Alterations of LDH and PAL coincided quite closely, and may have stemed from altered liver function. Gainer et al. believed that the elevated LDH was probably a direct result of LDH released from the lysed red blood cells.

MATERIALS AND METHODS

A. Experimental Animals

Horses of the Shetland and mixed Quarter breeds were purchased from a rancher in West Texas. The incidence of natural insect vectors is low in this area because of the low humidity and as a result the incidence of EIA in the equine population of that area is reduced. All of the horses purchased from that area of Texas have also been negative for EIA.

The animals were grouped into lots of six and underwent a round robin. The round robin consisted of transfusing each horse with 300 ml of whole blood obtained from another animal in the group. The horses were then observed for 90 days for clinical signs of EIA. These observations included taking rectal temperature twice daily throughout this period. The group of horses was considered free of the virus only after one of the 6 animals was injected with 300 ml of whole blood from a known carrier and clinical EIA developed in this animal. The remaining 5 negative animals in each of the group were subsequently employed in the infectivity trials.

All animals were fed a ration of Purina Horse Checkers supplemented with 1000 units of Vitamin D per lb of feed. The horses were fed at a rate of 1 1/4 lb of feed per 100 lb of body weight. Water was available ad libitum.

Experimental EIA was produced by inoculating the susceptible animals with 100 ml of whole blood via the jugular vein from a carrier. The horses were housed in individual screened stalls to eliminate the possibility of insect vectors spreading EIA to susceptible animals in the surrounding area.

B. Collection and Preparation of Blood

Blood samples were collected at three-day intervals via jugular venipuncture into 50 ml evacuated containers which contained 50 mg disodium ethylenediamine tetraacetic acid as an anticoagulant.

The blood samples were centrifuged at 2000 x g for 20 minutes at 4° C. Plasma was removed and stored in 50 ml glass vials after being passed under a stream of nitrogen. The erythrocytes were washed three times with physiological saline and the white blood cells were removed by aspiration. The pellet of erythrocytes that remained after the last washing was passed under a nitrogen stream and stored in a 50 ml glass vial. The plasma and erythrocyte samples were stored at -20° C until lipid extraction was initiated.

C. Lipid Extraction

All solvents used in this experiment were redistilled, with the exception of diethyl ether. Erythrocytes and plasma were extracted with 20 volumes of chloroform-methanol (2/1, v/v). In order to prevent clumping, erythrocytes were added slowly to cold methanol while being stirred with a magnetic stirrer. The mixture of erythrocytes and methanol was stirred

vigorously for about 5 minutes before chloroform was slowly added. The extracted mixtures of erythrocytes and plasma were filtered through 2 thicknesses of diethyl ether-extracted Whatman No. 1 filter paper. This filtrate was placed in a round-bottomed flask and concentrated at 65° C to about 30 ml on a rotary-vacuum evaporator (Wallace, 1966; Ways and Hanahan, 1964).

Approximately 5 grams of NaCl were placed in a separatory funnel which was wetted with chloroform to test for possible leaks. The sample of extracted lipid mixture was added to the separatory funnel and distilled water added to about 3/4 the volume of the funnel. This mixture was vigorously shaken and allowed to stand for 15 minutes to allow separation of the phases (Folch, 1957). The lower fraction (lipid-chloroform mixture) was drawn off into a flask containing anhydrous sodium sulfate. Three additional extractions were carried out on the material in the separatory funnel with 5 ml chloroform added prior to vigorous shaking.

The flask containing the lipid-chloroform mixture and anhydrous sodium sulfate was flushed with nitrogen and allowed to dry for 1 1/2 hours at 4° C.

D. Preparation of Methyl Esters

After drying, the lipid-chloroform mixture was placed in a 20 x 150 mm tube and evaporated to dryness under a stream of nitrogen in a water-bath at 70° C. Five ml of a transmethylation solution composed of 150 ml of dry methanol, 75 ml benzene and 7.5 ml concentrated sulfuric acid were added to each tube

(Wallace et al., 1965). The mixture was refluxed at approximately 120° C for 1 1/2 to 2 hours. Five ml of petroleum ether and 15 ml distilled water were added to each tube (Stoffel et al., 1959; Connellan, 1965). The tubes were vigorously shaken and allowed to stand until the petroleum ether and water phases had separated. The lower water phase was removed with a Pasteur pipette and the washing procedure repeated 3 times to remove all traces of acid. After the final wash the petroleum ether layer was removed and dried over anhydrous sodium sulfate under nitrogen for at least 1 1/2 hours. The methyl esters were subsequently concentrated by evaporation under nitrogen in a water-bath at about 65° C and drawn into a microliter syringe and injected into a gas chromatograph.

E. Gas-Liquid Chromatography of Acids

The gas-liquid chromatography phase of the study was carried out with a F & M Model 700 gas chromatograph. It was equipped with dual columns and dual flame ionization detectors. The columns were 10 feet in length x 1/4" (O.D.) and were packed with 8% polyethylene glycol adipate (EGA) on acid-washed Chromport (80-100 mesh). The columns were hand-packed by vibration and conditioned in the F & M Model 700 overnight at 195° C with a helium flow. The following morning the flow rate for the carrier gas (helium) was established at 60 ml per minute for each column. The flame ionization detectors were

supplied with a hydrogen and compressed air mixture. The hydrogen flow rate set at 18 pounds per square inch and the compressed air at 15 pounds per square inch. The injection port and detectors of the Model 700 were maintained at 275° C while the columns were held at 180 ° C (James, 1960).

F. Thin-Layer Chromatography to Separate Lipid Classes

Pyrex plates (200 x 200 mm) were washed, rinsed, oven-dried, and cleansed with methanol before use. A soupy suspension of Silica Gel G, prepared by mixing 22.5 gm with 50 ml of water, was spread over the glass plates with a Brinkmann adjustable applicator. The plates were gently shaken to thin thickened areas and allowed to dry for 30 minutes. They were then placed in an oven for 30 minutes at 115 ° C for activation prior to being used for lipid separation. The activated plates were removed from the oven and allowed to cool to ambient temperature. A lane of about 20 mm in width was marked on one side of the plate (Mangold, 1961). A standard mixture (Hormel Institute Standard Mixture II A) containing cholesterol, cholesterol oleate, triolein, oleic acid and hydrogenated lecithin was spotted about 15 mm from the bottom of the plate in the control lane. The lipid extract was concentrated in a water-bath to about 2 ml. This was drawn into a glass tuberculin syringe and spotted along a line 15 mm from the bottom of the plate. In order to regulate the size of the spots, a bent 22 gauge needle was employed.

The solvent system used to develop the TLC plates was composed of 126 ml petroleum ether, 22.5 ml diethyl ether, and 1.5 ml formic acid (Mangold, 1961). The solvent was poured into a Desaga chromatography tank. The paper wick along the back wall of the tank was soaked with solvent to insure a saturated atmosphere. The TLC plates were carefully placed in the tanks and developed until the solvent front was approximately 10 mm from the top of the plate. After development, the plate was placed under a stream of nitrogen to remove the solvents.

The lipid classes were visualized by spraying the TLC plate with 2'7' dichlorofluoresein and observing under long wave ultra-violet light. Bands corresponding to standard lipids were marked (Stahl, 1965). The bands were scraped from the TLC plate onto sheets of glassine weighing paper and then transferred to 20 x 150 mm tubes to which 5 ml of transmethylation mixture had been added. The material was processed in the same manner as that employed in esterifying the total lipid extract.

G. Method for Determining Fatty Acids

The method employed to determine the retention time of unknown fatty acid esters involved the passage of a standard ester through the gas chromatograph and comparison of the known retention times with the retention times of the unknown esters. The standard mixture contained the following fatty esters: C11:0, C12:0, C13:0, C14:0, C15:0, C16:0,

C17:0, C18:0, C18:1, C18:2, C20:0, C22:0, C22:1, C24:0, and C24:1. In order to determine the concentrations of the fatty acids the triangulation method was employed. The height and 1/2 of the width of the peak were employed to calculate peak area. The total area of all peaks was calculated and the relative percentage area of each peak was determined (James, 1960).

RESULTS AND DISCUSSION

The primary purposes of this investigation were: (1) to study the changes in the fatty acids of the total lipids and the fatty acids of lipid classes in the plasma and erythrocytes of horses infected with EIA and (2) to determine if the lipemia and consequently the lipids or fatty acids present in the blood could be responsible for the hemolytic anemia. It has been established that various lipids can produce hemolysis *in vivo* (Blass and Dean, 1966).

Due to the lack of information in the literature relating to this investigation, an initial study was required to determine the ranges of total fatty acid values of normal horse blood. Once this was accomplished, it was possible to study the alterations which occurred in the blood lipids of EIA-infected horses.

A. Establishment of Pre-Inoculation Values

The initial investigation provided data on the ranges in relative concentrations of fatty acids in the total lipids of plasma and erythrocytes of horses before inoculation with EIA virus.

The major fatty acids in the plasma lipids of horses before infection were C16:0, C16:1, C18:0, C18:1, C18:2 and

C18:3. Figures 1 and 2 represent data on body temperature and relative percent composition of fatty acids present in the total lipids of plasma obtained from Horse No. 3 for 19 days before inoculation with EIA virus. During this 19-day period, C16:0 and C18:0 had a concentration range of 13 to 17 percent. C16:1 and C18:3 had a range of 1.5 to 4.5 percent concentration. C18:2 showed the highest concentration of any fatty acid in the total lipids of plasma in the horse prior to infection. It ranged between 34.87 and 43.39 percent. Investigations to determine the concentrations of fatty acids in plasma were extended to 48 days before inoculation in other animals, and similar results were obtained as shown in Tables 1 and 3. These data are felt to be representative of the fatty acids in plasma of normal horses on the feed ration outlined in Materials and Methods, Section I.

The second part of the initial investigation involved determining the ranges in concentrations of fatty acids in the total lipids of erythrocytes of horses prior to inoculation. Figures 3 and 4 represent the data collected for a period of 19 days before infection. The major fatty acids in the erythrocytic lipids of Horse No. 3 were C16:0, C16:1, C18:0, C18:1, C18:2 and C24:0.

The major fatty acid in the erythrocytes was C16:0 which comprised about 25 percent of the total lipid extract (Table 2). C18:0 and C18:1 made up approximately 31-38 percent and C18:2 and C16:1 about 3 percent of the total lipids in the erythrocyte as shown in Table 2. There was

Figure 1. Plots of body temperature and percent of C18:1 and C18:2 of the fatty acids in the total lipid extract of plasma from Horse No. 3.


TEMPERATURE °F
104
103
102
101
100
99
98
% CONCENTRATION
50
40
30
20
10
0
C 18:2
C 18:1
19 16 12 8 0 2 6 9 13 16 20 23 27 30
DAYS BEFORE INOCULATION
DAYS AFTER INOCULATION

Figure 2. Plots of body temperature and percent of C16:0, C16:1, C18:0, and C18:3 of the fatty acids in the total lipid extract of plasma from Horse No. 3.

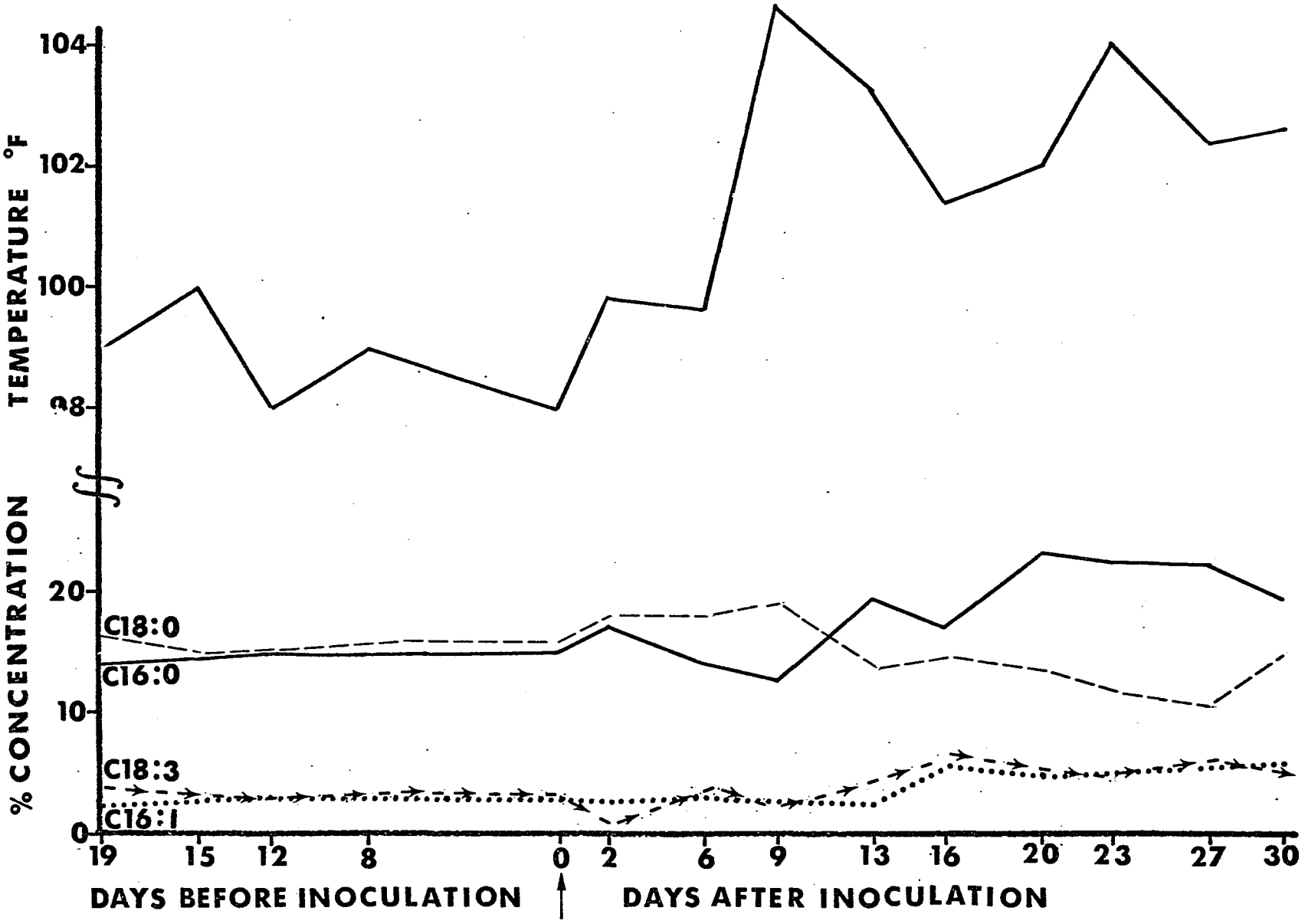

104
102
100
98
TEMPERATURE °F
% CONCENTRATION
20
10
0
C18:0
C16:0
C18:3
C16:1
19
15
12
8
0
2
6
9
13
16
20
23
27
30
DAYS BEFORE INOCULATION
DAYS AFTER INOCULATION

Table 1. Percent of individual fatty acids of the total lipid extract from plasma of Horse No. 3.

Fatty Acid	Days Before Inoculation					Days After Inoculation								
	19	11	7	3	0	3	7	10	14	17	21	24	28	31
C14:0	0.72	0.99	0.87	0.90	1.00	0.81	0.92	0.52	Tr[a]	1.20	1.15	1.19	1.24	1.33
C16:0	13.91	14.42	14.90	14.63	14.99	17.02	13.98	12.81	19.20	17.68	23.30	21.67	21.93	19.41
C16:1	1.94	2.45	2.92	2.76	2.73	2.37	3.06	2.46	3.27	5.23	4.66	5.05	5.29	5.24
C18:0	16.33	14.71	14.90	15.62	15.75	17.78	17.57	18.62	13.66	14.43	13.22	11.55	10.18	14.40
C18:1	15.44	24.87	17.41	15.55	16.75	14.18	16.24	12.81	18.45	22.79	23.13	23.39	26.06	25.81
C18:2	43.39	34.87	40.29	41.72	40.36	42.27	39.51	45.17	34.86	27.63	25.72	27.18	23.77	25.75
C18:3	3.52	2.91	2.82	3.12	3.13	0.49	3.17	1.89	4.17	6.54	5.08	4.69	5.77	5.12
C20:0	0.41	0.41	0.50	0.34	0.34	0.38	0.51	0.49	0.24	0.49	0.15	0.19	0.24	0.16
C20:1	0.40	0.89	0.59	0.36	0.37	0.56	0.47	0.44	0.33	0.41	0.47	0.37	0.45	0.35
C21:1	-	0.23	-	-	-	-	-	-	-	-	-	-	-	-
C20:4	0.59	0.55	0.90	0.59	0.85	0.53	0.53	0.60	1.19	0.45	0.58	1.83	1.98	0.51
C22:0	0.29	0.41	0.59	1.08	0.21	0.32	0.59	0.31	0.28	0.18	0.19	0.22	0.17	0.10
C22:1	Tr	Tr	Tr	Tr	Tr	0.11	Tr	Tr	2.51	0.37	Tr	Tr	Tr	Tr
C24:0	0.30	Tr	Tr	0.34	0.08	0.38	0.23	0.49	0.14	0.50	0.53	Tr	Tr	Tr
C24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S/U[b]	0.48	0.46	0.49	0.51	0.50	0.61	0.54	0.52	0.52	0.54	0.65	0.56	0.53	0.56

[a]Trace amount.

[b]Saturated to unsaturated fatty acid ratio.

Table 2. Percent of individual fatty acids of the total lipid extract from erythrocytes of Horse No. 3.

Fatty Acid	Days Before Inoculation				Days After Inoculation								
	19	11	8	0	2	6	9	13	16	20	23	27	30
C14:0	0.70	0.47	0.43	0.69	0.71	0.50	0.42	0.31	0.63	0.42	0.39	0.90	0.74
C16:0	24.40	20.90	20.75	21.40	23.57	19.45	21.57	5.60	23.30	16.77	17.16	19.82	21.47
C16:1	2.84	1.94	1.58	1.80	3.13	1.55	1.60	0.91	2.53	1.50	1.89	1.62	1.69
C18:0	21.32	19.32	19.71	19.86	21.00	17.21	16.73	3.95	24.57	19.98	17.63	18.80	22.30
C18:1	14.84	12.10	18.57	18.39	21.54	16.98	14.74	4.37	19.29	15.04	15.04	14.31	15.62
C18:2	1.65	1.94	2.66	1.64	3.18	1.79	1.26	3.49	1.65	1.40	2.12	3.43	1.56
C18:3	Tr[a]	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.25	Tr	Tr	Tr	Tr
C20:0	1.40	6.46	1.71	0.59	0.56	0.45	0.53	Tr	0.25	0.28	0.70	0.22	0.20
C20:1	1.77	2.12	1.08	0.95	1.00	0.88	1.14	3.41	1.09	0.90	0.73	0.16	1.10
C21:0	0.98	1.47	2.03	1.07	1.08	1.22	0.92	1.06	0.67	0.71	1.19	0.38	0.67
C21:1	1.83	4.68	4.15	3.98	3.96	3.40	3.58	1.42	4.26	3.25	2.27	3.00	3.32
C20:4	1.46	2.18	0.80	1.54	0.64	0.50	0.74	4.22	0.84	0.42	0.45	1.31	0.54
C22:0	1.83	1.94	1.29	1.37	1.98	1.54	2.08	9.86	1.90	1.53	1.21	3.27	2.07
C24:0	5.49	4.71	4.27	6.11	5.87	4.79	5.40	3.27	7.25	5.34	5.10	3.77	3.98
C24:1	1.08	9.67	5.23	5.36	8.33	10.14	6.36	7.34	7.64	7.16	12.19	4.69	6.43
C24:U1	13.27	7.06	12.63	7.55	Tr	7.67	10.58	38.03	1.06	2.54	4.17	4.35	6.66
C24:U2	-	-	-	-	-	-	-	-	0.00	6.35	14.45	15.28	3.11
S/U[b]	1.44	1.32	1.07	1.23	1.31	1.05	1.19	0.38	1.51	1.16	0.81	0.97	1.28

[a] Trace amount.

[b] Saturated to unsaturated fatty acid ratio.

Table 3. Percent of individual fatty acids of the total lipid extract from erythrocytes of Horse No. 9.

Fatty Acid	Days Before Inoculation						Days After Inoculation					
	48	46	43	7	4	0	3	7	10	14	17	20
C14:0	3.67	0.74	1.27	2.15	0.74	0.68	2.46	1.29	1.10	1.22	1.65	0.82
C16:0	15.35	16.50	16.99	21.19	25.54	24.71	20.60	20.94	14.30	13.25	26.68	25.42
C16:1	4.34	1.65	1.36	2.58	1.69	1.51	7.09	2.36	4.70	1.74	2.48	1.58
C18:0	14.99	16.50	14.87	17.67	19.35	19.91	13.87	17.04	14.10	22.60	22.59	36.77
C18:1	15.04	16.31	13.25	15.20	17.56	17.20	15.59	14.24	23.20	25.50	23.37	21.11
C18:2	3.30	2.44	0.93	3.83	1.13	1.80	1.46	1.68	0.70	0.54	2.20	1.01
C18:3	2.27	1.36	1.85	1.75	1.33	1.49	0.92	1.22	8.00	5.70	0.55	0.19
C20:0	1.85	1.11	0.64	2.68	2.06	1.98	1.51	2.06	7.20	1.37	9.30	1.20
C20:1	3.56	2.27	1.44	3.35	0.90	1.00	1.05	0.42	1.10	5.19	0.49	0.31
C21:0	3.92	2.19	2.21	1.15	2.35	3.22	1.87	1.74	1.40	1.74	1.60	0.50
C20:4	1.55	1.03	1.15	3.81	1.38	1.65	0.46	0.35	1.80	3.62	-	0.63
C22:0	9.66	15.55	12.70	8.92	-	-	0.74	0.72	3.10	1.11	-	2.21
C24:0	1.70	1.69	1.43	1.89	5.61	5.26	2.74	2.87	0.70	2.96	3.97	3.93
C24:1	8.73	17.54	17.56	-	11.94	13.21	6.98	6.65	1.20	-	-	-
S/U[a]	1.32	1.27	1.37	1.76	1.55	1.47	1.31	1.73	1.01	1.05	2.26	2.85

[a]Saturated to unsaturated fatty acid ratio.

evidence that the erythrocytes contained longer chain fatty acids than the plasma as seen in Table 2 and that the erythrocytes contained about 5 percent of C24:0. Table 3 gives supporting data which represent a 48-day testing period prior to infection of Horse No. 9 with EIA virus.

B. Changes in Fatty Acids of Total Lipid Extract in Experimental EIA

The horses were injected with 100 ml of whole citrated blood from a known EIA carrier. One day after inoculation the body temperature of Horse No. 3 became elevated and did not return to the normal range. It died after 30 days. Figures 1 and 2 show the changes that occurred in the plasma during the course of experimental EIA of Horse No. 3. The largest changes were found in C18:1 and C18:2. C18:2 had an almost equal and opposite change to C18:1 (Figure 2). Nine days after inoculation Horse No. 3 had a temperature of almost 105°F, the highest recorded, and C18:2 reached its maximum concentration of 45.17 percent in the plasma. C18:2 dropped from 45.17 percent on day nine to 25.72 percent on day 20 after inoculation. Twenty days after inoculation the concentration of C18:2 dropped for the first time to a lower percentage than C18:1. C16:0 became elevated to 23.3 percent at this time and remained elevated until death. As the concentration of C18:0 increased the concentration of C16:0 dropped but to a lesser degree as shown in Figure 2. C16:1 and C18:3 became elevated to about twice their initial concentrations by day 16.

Other fatty acids present in the plasma included C14:0, C20:0, C20:1, C21:0, C21:1, C22:0, C22:1, C24:0 and C24:1. Since their values were usually less than 1 percent and no marked changes were noted, they were not considered significant. The data are listed in Table 1.

The fatty acid alterations in the erythrocytes were more drastic than the changes in the plasma. Figures 3 and 4 show that almost all of the major fatty acids increased slightly with the exception of C24:0 which dropped from about 10 percent to zero percent in 2 days. Between days 2 and 9 after inoculation there was a small decrease in the concentrations of C16:0, C18:0, and C18:1. From day 9 to day 13, all three components decreased to about one-fourth of their preinoculation levels. The drastic decrease in these three fatty acids of the erythrocyte was offset by an increase in C24 fatty acids of unknown saturation and/or branching (C24:U1) as shown in Figure 4. Two days after inoculation C24:U1 disappeared but by day 13 it increased to 38.03 percent of the total erythrocytic lipids in Horse No. 3. By day 16 C24:U1 dropped to less than 30 percent and remained there until the horse died. C24 fatty acids of unknown saturation and branching (C24:U2) became elevated to about 15 percent but decreased to less than 5 percent before death.

Figure 5 represents the alterations in the ratio of saturated to unsaturated fatty acids during the course of experimental EIA. The ratio of saturated to unsaturated fatty

Figure 3. Percent of C16:0, C18:0, C18:1, and C18:2 of the fatty acids in the total lipid extract of erythrocytes from Horse No. 3.

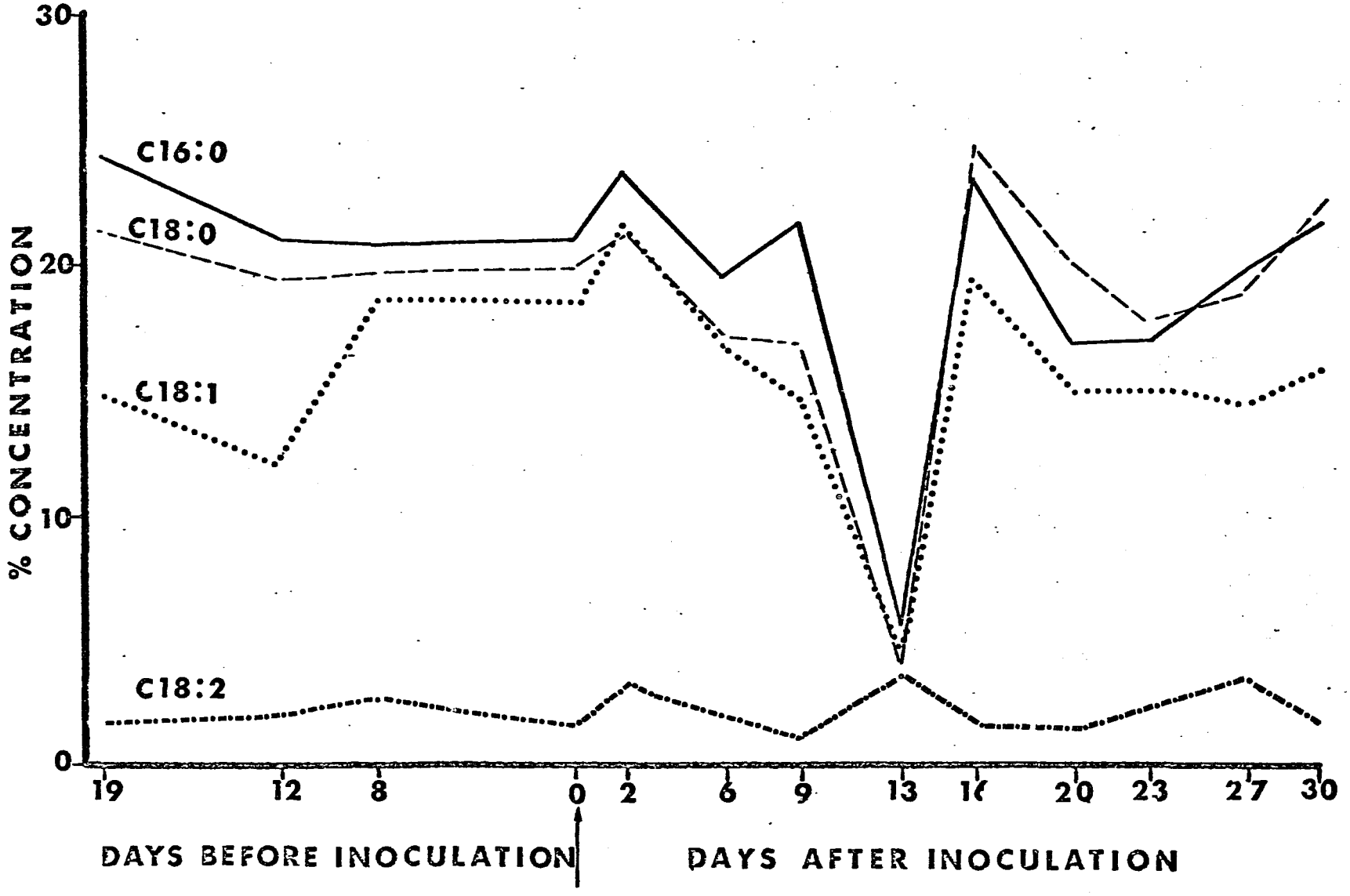

30
20
10
0
% CONCENTRATION
C16:0
C18:0
C18:1
C18:2
19
12
8
0
2
6
9
13
16
20
23
27
30
DAYS BEFORE INOCULATION
DAYS AFTER INOCULATION

Figure 4. Plots of body temperature and percent of C16:1, C24:U1, and C24:U2 of the fatty acids in the total lipid extract of erythrocytes from Horse No. 3.

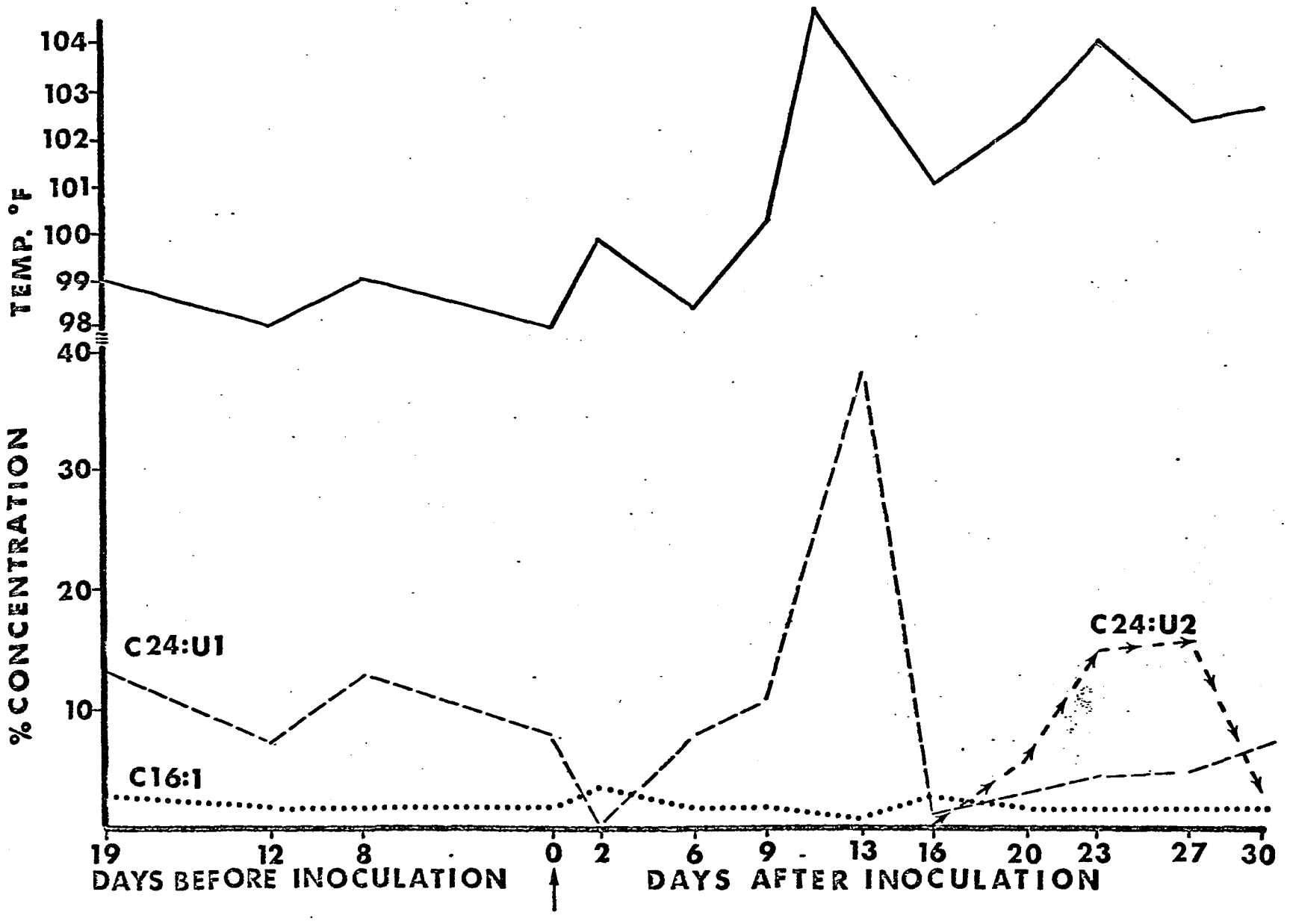
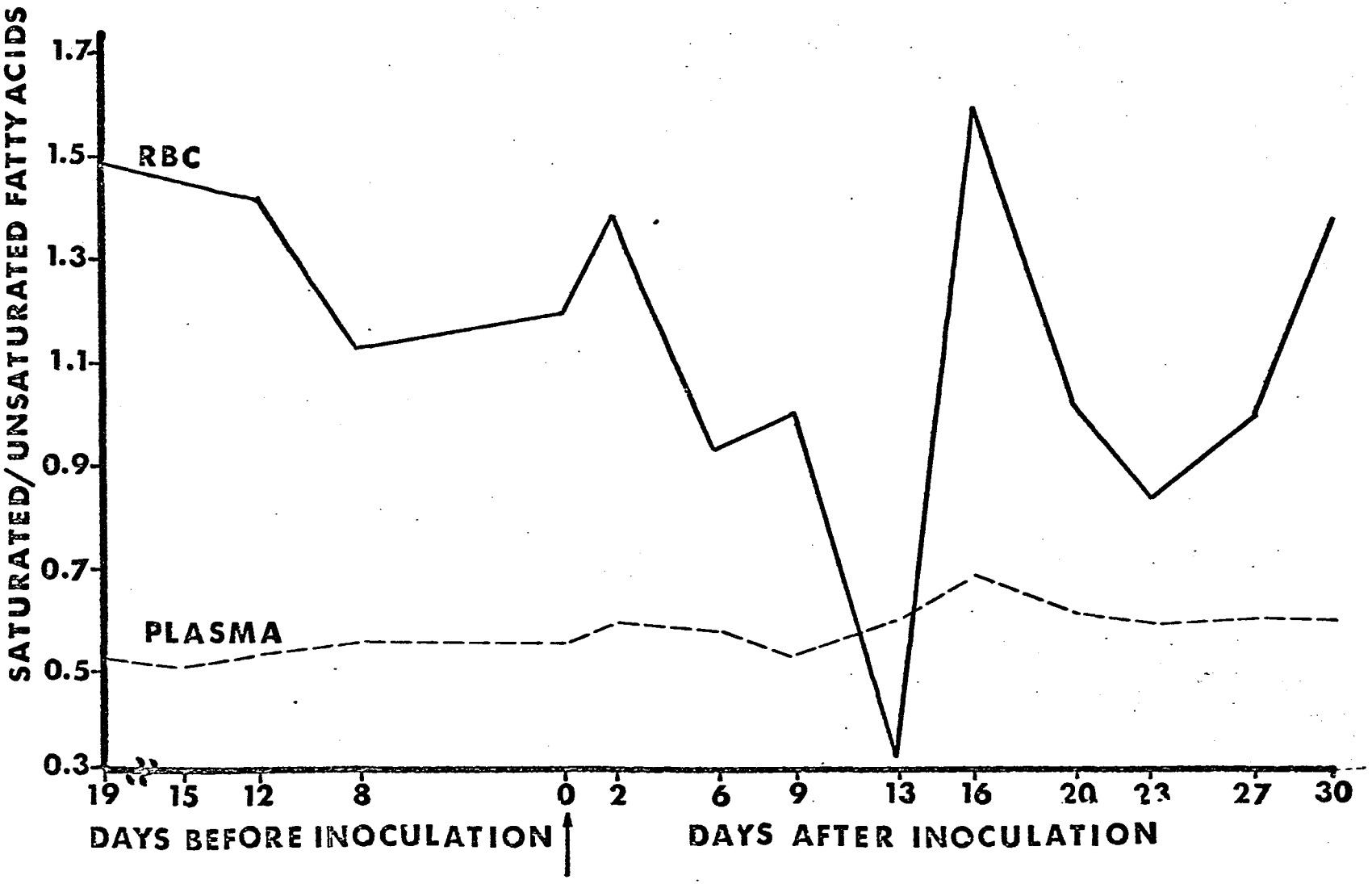

104
103
102
101
100
99
98
TEMP. °F
40
30
20
10
% CONCENTRATION
C24:U1
C16:1
C24:U2
19
12
8
0
2
6
9
13
16
20
23
27
30
DAYS BEFORE INOCULATION
DAYS AFTER INOCULATION

Figure 5. Ratio of saturated to unsaturated fatty acids of the total lipid extract of the plasma and erythrocytes from Horse No. 3.


SATURATED/UNSATURATED FATTY ACIDS
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0.3
RBC
PLASMA
19
15
12
8
0
2
6
9
13
16
20
23
27
30
DAYS BEFORE INOCULATION
DAYS AFTER INOCULATION

acids was relatively constant in the plasma throughout the course of the disease. The erythrocytes of Horse No. 3 shifted markedly from day 9 to day 13 after inoculation and was due to the decrease in the concentrations of C16:0 and C18:0 and the large increase in the C24:U1. The saturated fatty acids had greatly increased over the unsaturated fatty acids by day 16 after inoculation. The saturated acids later decreased below preinoculation values and at death returned to the initial range. Horse No. 11 survived an experimental infection of EIA, and did not exhibit an increase in the unsaturated fatty acid concentration as shown by Horse No. 3 which died from acute EIA.

Horse No. 11 survived the experimental infection and exhibited clinical symptoms of EIA. Body temperature peaked at 104.5°F 9 days after infection until day 20 at which time it returned to a normal range. It remained normal until the experiment was terminated 62 days after infection (Figure 6, Table 4).

The fatty acids of the total lipid-extract of the plasma of Horse No. 11 had patterns almost identical with the animals that died from the disease. C18:2 increased slightly for 9 days after infection and then declined to a range less than half of its initial value 18 days after infection. C18:2 returned to a normal range on day 22 and remained at this area until the experiment was terminated at 62 days. The concentration of C18:1 (Figure 6 and Table 4) exhibited an almost equal and opposite change as seen for C18:2 throughout the course of the experiment.

Figure 6. Plots of body temperature and percent of C18:1 and C18:2 of the fatty acids in the total lipid extract of plasma from Horse No. 11.

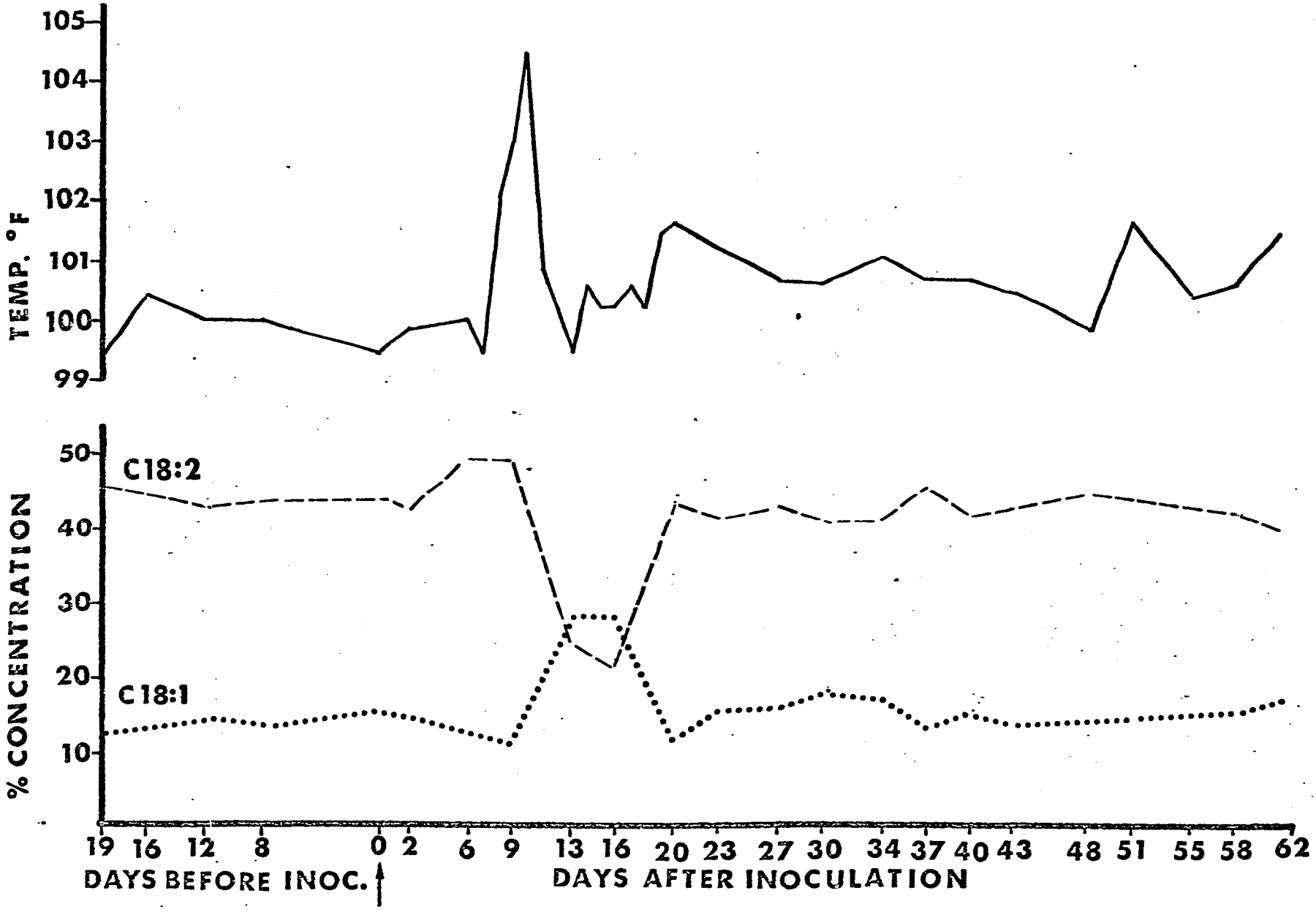

105
104
103
102
101
100
99
TEMP. °F
50
40
30
20
10
% CONCENTRATION
C18:2
C18:1
19 16 12 8 0 2 6 9 13 16 20 23 27 30 34 37 40 43 48 51 55 58 62
DAYS BEFORE INOC.
DAYS AFTER INOCULATION

Table 4. Percent of individual fatty acids of the total lipid extract from plasma of Horse No. 11.

	Days	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C21:0	C20:4	C22:0	C22:1	C24:0	S/U[a]
Days Before Inoculation	19	0.43	13.56	1.39	20.68	12.22	45.83	1.03	0.45	0.26	0.24	0.71	0.15	0.20	0.76	0.59
	16	0.53	13.29	1.47	19.60	13.29	44.32	1.01	0.41	0.30	0.30	0.79	0.15	0.61	1.75	0.58
	12	0.59	13.93	1.50	18.78	14.59	42.77	1.26	0.42	0.30	0.23	0.92	0.23	0.41	1.96	0.59
	8	0.55	13.77	1.46	19.14	13.94	43.49	0.88	0.45	0.38	0.24	0.97	0.21	0.21	2.25	0.60
Days After Inoculation	0	0.58	13.10	1.70	19.60	15.31	43.53	1.03	0.50	0.57	0.35	0.98	0.11	Tr	0.39	0.55
	2	0.58	12.93	1.44	19.69	14.13	42.24	1.81	0.42	0.35	0.29	1.03	0.93	0.67	1.77	0.59
	6	0.77	13.50	2.88	13.82	12.54	48.82	2.05	1.28	0.38	0.19	0.51	Tr	Tr	Tr	0.44
	9	0.63	13.74	2.06	17.77	11.02	48.67	1.22	0.36	0.21	0.17	1.03	0.32	0.13	Tr	0.51
	13	1.35	12.85	7.89	10.60	27.62	24.41	8.78	0.10	0.31	0.30	0.78	0.10	0.48	1.23	0.38
	16	2.02	18.88	7.66	8.83	27.89	20.83	9.19	0.05	0.24	0.18	0.36	0.07	0.47	0.58	0.46
	20	0.56	15.58	2.58	17.49	11.77	43.05	1.01	0.34	0.45	0.67	2.24	Tr	Tr	Tr	0.57
	23	0.52	12.75	4.56	18.07	15.36	41.07	2.12	0.33	0.30	0.25	1.00	0.23	0.14	0.70	0.51
	27	0.58	13.30	2.22	17.61	15.79	42.29	1.80	0.38	0.45	0.38	0.99	0.37	0.34	0.64	0.52
	30	0.71	11.52	2.52	18.59	17.31	40.26	2.17	0.41	0.54	0.38	1.28	0.23	0.15	1.03	0.51
	34	0.62	13.97	2.36	16.60	16.66	40.79	1.84	0.35	0.41	0.31	1.09	0.81	0.24	1.40	0.54
	37	0.55	12.79	2.04	15.49	13.12	45.26	1.60	0.33	0.29	0.44	1.06	2.00	0.87	1.64	0.52
	40	0.67	13.67	2.40	18.19	14.98	41.52	1.54	0.33	0.46	0.34	1.21	0.35	0.31	1.94	0.57
	43	0.50	13.74	2.17	18.53	13.34	42.38	1.80	0.31	0.36	0.29	1.30	0.43	1.18	1.30	0.56
	48	0.51	13.29	2.21	19.41	13.72	44.02	1.41	0.29	0.27	0.26	1.23	0.57	0.18	0.56	0.55
	51	0.50	13.30	1.85	18.30	14.25	43.84	1.63	0.38	0.33	0.32	1.36	0.35	0.31	0.83	0.53
	55	0.59	13.16	2.32	18.59	14.4	42.52	1.64	0.37	0.37	0.25	1.49	0.54	0.84	0.59	0.54
	58	0.59	13.93	2.36	18.43	14.86	41.81	1.56	0.41	0.36	0.31	1.13	0.48	0.31	1.24	0.57
	62	0.70	14.02	2.88	17.40	16.18	39.78	2.42	0.31	0.35	0.29	1.34	0.50	0.48	1.06	0.54

[a]Saturated to unsaturated fatty acids.

[b]Trace amount.

Concentrations of C16:0 and C18:0 from Horse No. 11 were similar to those of Horse No. 3 with one notable exception. C16:0, which remained elevated in the plasma of the horses which died of EIA returned to normal in Horse No. 11 by day 22. The greatest changes which occurred in the plasma of the surviving horse which recovered were C16:1 and C18:3. These two fatty acids increased three-fold by 13 days after infection and remained elevated for 4 days, at which time they began to decline and reached a normal range on day 20 as shown in Table 4.

The changes in fatty acids of erythrocytes of Horse No. 11 exhibited similar alterations in C18:0, C18:1, and C18:2 as did Horse No. 3. There was one notable difference in the fatty acids of erythrocytic lipids of horses that died as a result of the disease and those which survived. Horse No. 3 in the acute stage of EIA had about a three-fold decrease in the concentration of C16:0. Horse No. 11 had a relatively constant concentration of C16:0 throughout the course of the disease (Table 5).

C. Fatty Acids of Lipid Classes of Plasma

Thin-layer chromatography was employed to separate the lipid classes in order to obtain data on the fatty acids of the individual classes of lipids by GLC. The TLC method separated the lipids into five classes: PL, sterols, FFA, TG, and SE. All classes of lipids were analyzed for fatty acid composition except the sterol fraction.

Table 5. Percent of individual fatty acids of the total lipid extract from erythrocytes of Horse No. 11.

Fatty Acid	Days After Inoculation									
	0	3	7	11	19	21	25	28	32	34
C14:0	1.26	0.76	0.74	0.74	0.65	0.27	0.66	0.46	1.97	0.47
C16:0	20.96	23.00	24.72	23.88	26.62	17.89	22.45	15.94	26.40	12.12
C16:1	1.98	2.16	6.00	1.88	1.31	1.47	2.08	1.79	6.93	1.80
C18:0	19.54	20.76	23.08	13.22	24.18	14.03	21.86	15.93	20.67	10.60
C18:1	18.44	21.11	8.34	11.58	20.46	11.51	21.16	13.73	23.17	7.72
C18:2	13.87	2.55	2.47	2.76	1.91	1.45	2.11	1.81	11.61	7.67
C18:3	0.09	0.07	0.87	0.07	-	1.10	-	-	0.63	0.27
C20:0	0.88	0.93	0.72	0.61	0.67	-	0.92	0.48	0.19	1.50
C20:1	1.48	1.62	1.43	1.04	1.31	0.78	1.06	0.99	0.12	3.66
C21:0	1.26	1.62	2.12	1.76	2.29	0.48	1.50	0.80	0.08	3.38
C20:4	0.25	0.19	0.66	2.33	2.31	1.49	1.64	1.04	0.12	6.25
C22:0	1.82	1.91	2.41	2.24	0.11	-	0.52	-	-	1.46
C24:0	6.39	7.03	7.93	9.17	8.00	-	3.50	5.07	-	13.51
C24:1	6.68	10.62	7.44	4.89	3.62	-	14.84	4.94	-	10.26
S/U[a]	1.22	1.46	2.27	2.10	2.02	1.83	1.20	1.59	1.16	1.10

[a]Saturated to unsaturated fatty acid ratio.

Blood samples were taken at three- to four-day intervals and separated into plasma and washed erythrocytes prior to total lipid extraction and separation of lipid classes.

Analyses of the plasma PL fraction (Figure 7, Table 6) showed that C18:0 and C18:2 comprised approximately 65 percent of the fatty acids. Changes in the percentage composition of C16:0 and C18:1 tended to parallel one another. There appeared to be little correlation between the alterations in the major fatty acids of the PL fraction in plasma and the body temperature of the horse. There were no radical changes in the PL fraction of the plasma lipids until the disease became terminal. The saturated to unsaturated fatty acid ratio of the PL fraction of the plasma throughout the disease was relatively stable; only on day 20 did the percentages of saturated fatty acids exceed those of the unsaturated fatty acids.

The FFA fraction (Table 7) of the plasma was composed chiefly of unsaturated fatty acids. At no time did the saturated fatty acid percentage composition exceed that of the unsaturated fatty acids of the FFA fraction of the plasma. Seven days after infection C18:1 decreased markedly while C18:3 and C21:0 had noticeable increases; however, by day 10 all 3 of these fatty acids had returned to their normal ranges.

The major fatty acids of the TG fraction of the plasma were C16:0, C16:1, C18:1, and C18:2 with C14:0, C18:0, and C18:3 accounting for moderate percentages (Table 8 and Figure 8). The data in Figure 8 demonstrated that C18:1, C18:2, and

Figure 7. Plots of body temperature and percent of C16:0, C16:1, C18:0, C18:1, and C18:2 of the fatty acids in the phospholipid class of plasma from Horse No. 6.

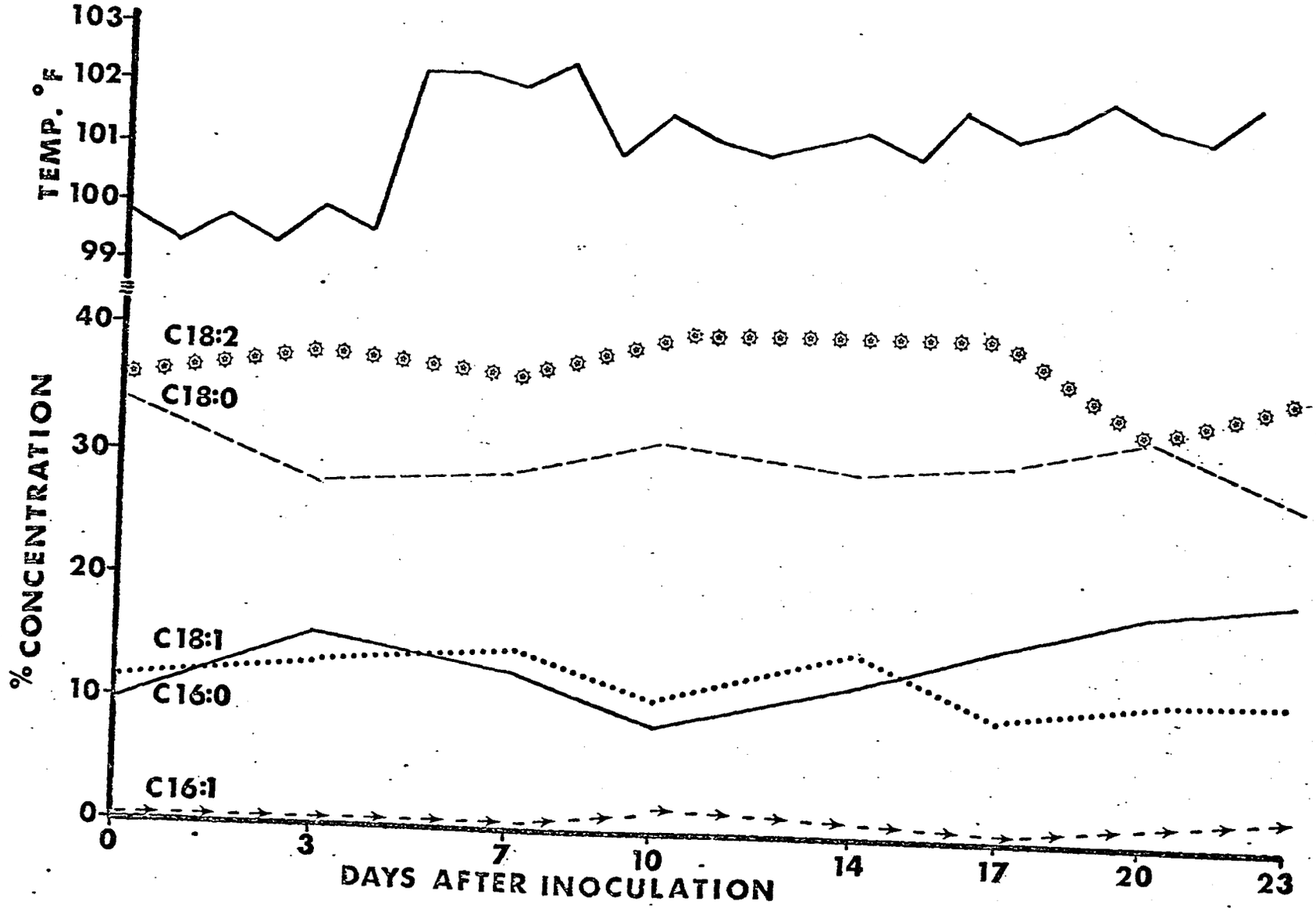

TEMP. °F
103
102
101
100
99
40
30
20
10
0
% CONCENTRATION
C18:2
C18:0
C18:1
C16:0
C16:1
0
3
7
10
14
17
20
23
DAYS AFTER INOCULATION

Table 6. Percent of individual fatty acids of the phospholipid fraction from plasma of Horse No. 6.

Fatty Acid	Days After Inoculation							
	0	3	7	10	14	17	20	23
C14:0	0.34	0.06	0.19	0.20	0.14	0.12	0.17	-
C16:0	9.99	15.53	12.71	8.49	11.98	15.28	18.39	19.84
C16:1	0.62	0.90	0.63	1.70	1.04	0.57	1.62	2.11
C18:0	34.42	27.63	28.93	31.41	29.38	30.24	32.61	27.74
C18:1	11.33	13.28	14.59	10.79	14.92	9.72	11.08	11.57
C18:2	35.76	38.11	36.53	40.02	40.20	40.12	32.58	36.27
C18:3	0.48	0.30	0.40	0.52	0.22	0.54	0.25	0.45
C20:0	0.51	0.50	0.67	0.37	0.08	0.18	0.31	0.28
C20:1	0.41	0.45	0.45	0.33	0.29	0.22	0.25	0.23
C21:0	0.35	0.18	0.28	0.31	0.19	0.08	0.06	-
C21:1	0.98	0.37	0.64	0.90	0.71	-	-	0.31
C20:4	0.43	0.24	0.34	0.42	-	0.24	0.27	0.21
C22:0	0.31	0.18	0.63	0.41	-	0.11	0.17	-
C22:1	0.72	0.48	1.15	0.78	-	-	0.05	-
S/U[a]	0.90	0.81	0.79	0.74	0.72	0.89	1.12	0.93

[a] Saturated to unsaturated fatty acid ratio.

Figure 8. Plots of body temperature and percent of C14:0, C16:0, C18:0, C18:1, and C18:2 of the fatty acids in the triglyceride class of plasma from Horse No. 6.

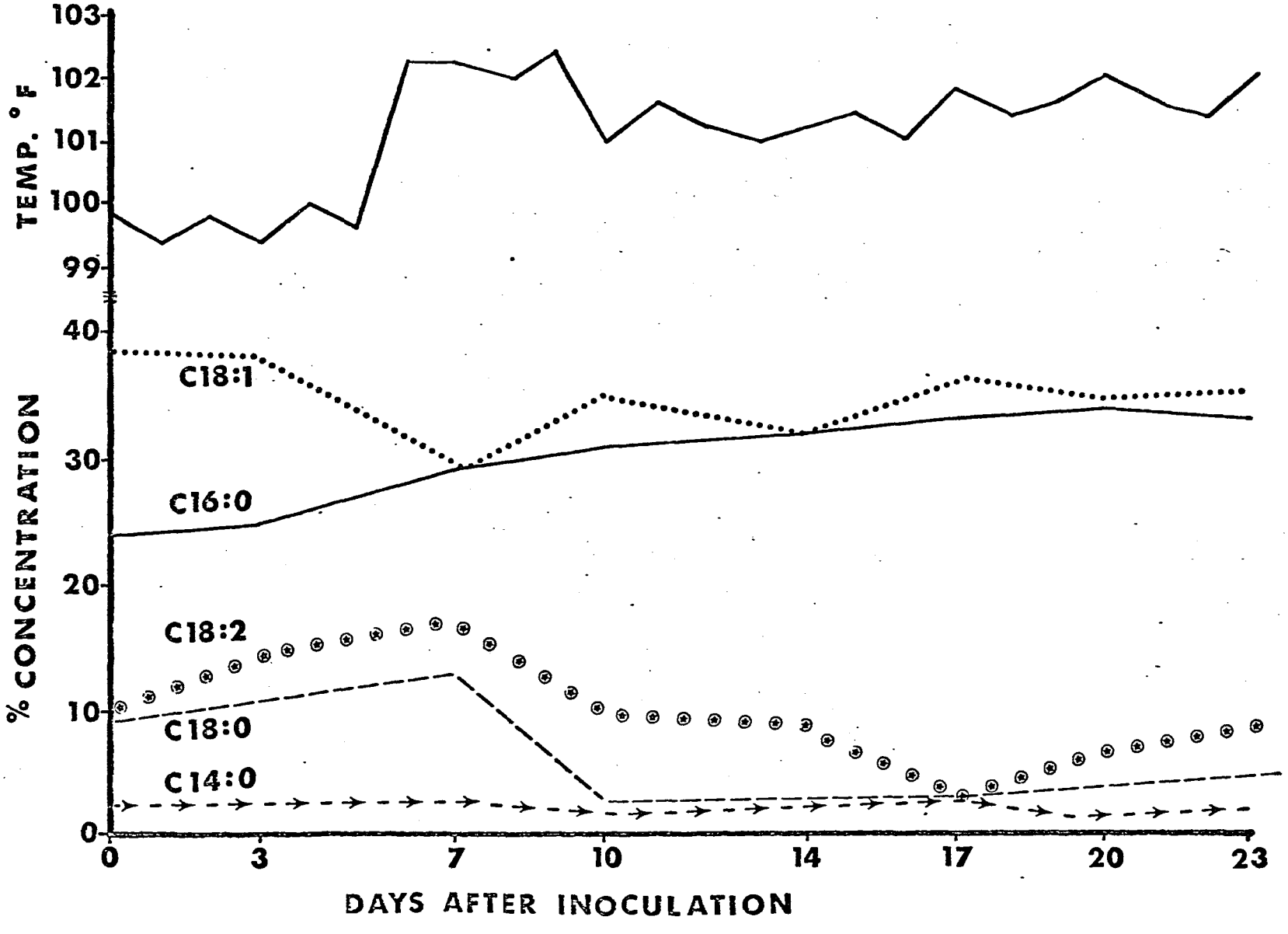

TEMP. °F
103
102
101
100
99
40
30
20
10
0
% CONCENTRATION
C18:1
C16:0
C18:2
C18:0
C14:0
0
3
7
10
14
17
20
23
DAYS AFTER INOCULATION

Table 7. Percent of individual fatty acids of the free fatty acid fraction from plasma of Horse No. 6.

Fatty Acid	Days After Inoculation 3	7	10	14
C14:0	1.70	1.09	1.71	4.23
C16:0	12.08	6.42	20.73	16.69
C16:1	2.10	1.85	10.16	12.39
C18:0	16.37	3.13	16.84	7.11
C18:1	31.87	5.44	31.55	36.77
C18:2	25.39	19.81	10.10	11.23
C18:3	-	25.88	6.11	8.76
C20:0	1.58	2.92	0.07	0.09
C20:1	0.28	2.44	0.38	0.35
C21:0	1.63	23.56	0.02	0.03
C21:1	0.26	-	0.10	0.31
C20:4	0.14	6.06	-	-
S/U [a]	0.55	0.60	0.67	0.40

[a]Saturated to unsaturated fatty acid ratio

Table 8. Percent of individual fatty acids of the triglyceride fraction from plasma of Horse No. 6.

Fatty Acid	Days After Inoculation							
	0	3	7	10	14	17	20	23
C14:0	2.63	2.53	2.52	1.56	2.26	2.64	1.24	1.87
C16:0	24.17	24.67	29.06	31.10	31.84	33.01	33.97	32.87
C16:1	6.36	4.00	3.45	11.61	10.83	11.62	13.65	8.54
C18:0	9.32	10.40	12.17	2.97	2.28	3.00	3.59	4.60
C18:1	38.74	37.80	28.89	35.10	32.14	36.41	34.60	35.19
C18:2	10.13	14.50	17.05	9.37	8.66	3.42	6.66	8.66
C18:3	3.96	0.64	4.22	4.95	9.76	8.19	6.08	7.76
C20:0	0.10	3.20	0.16	0.04	0.23	0.24	Tr[a]	-
C20:1	0.76	0.11	0.49	0.29	0.07	0.06	0.20	-
C21:0	0.17	0.91	0.27	0.03	Tr	Tr	Tr	-
C21:1	0.36	0.38	0.38	0.19	0.18	0.26	Tr	-
C20:4	-	0.36	-	-	-	-	-	-
C22:0								
C22:1								
S/U[b]	0.60	0.72	0.81	0.58	0.59	0.65	0.63	0.65

[a] Trace amount.

[b] Saturated to unsaturated fatty acid ratio.

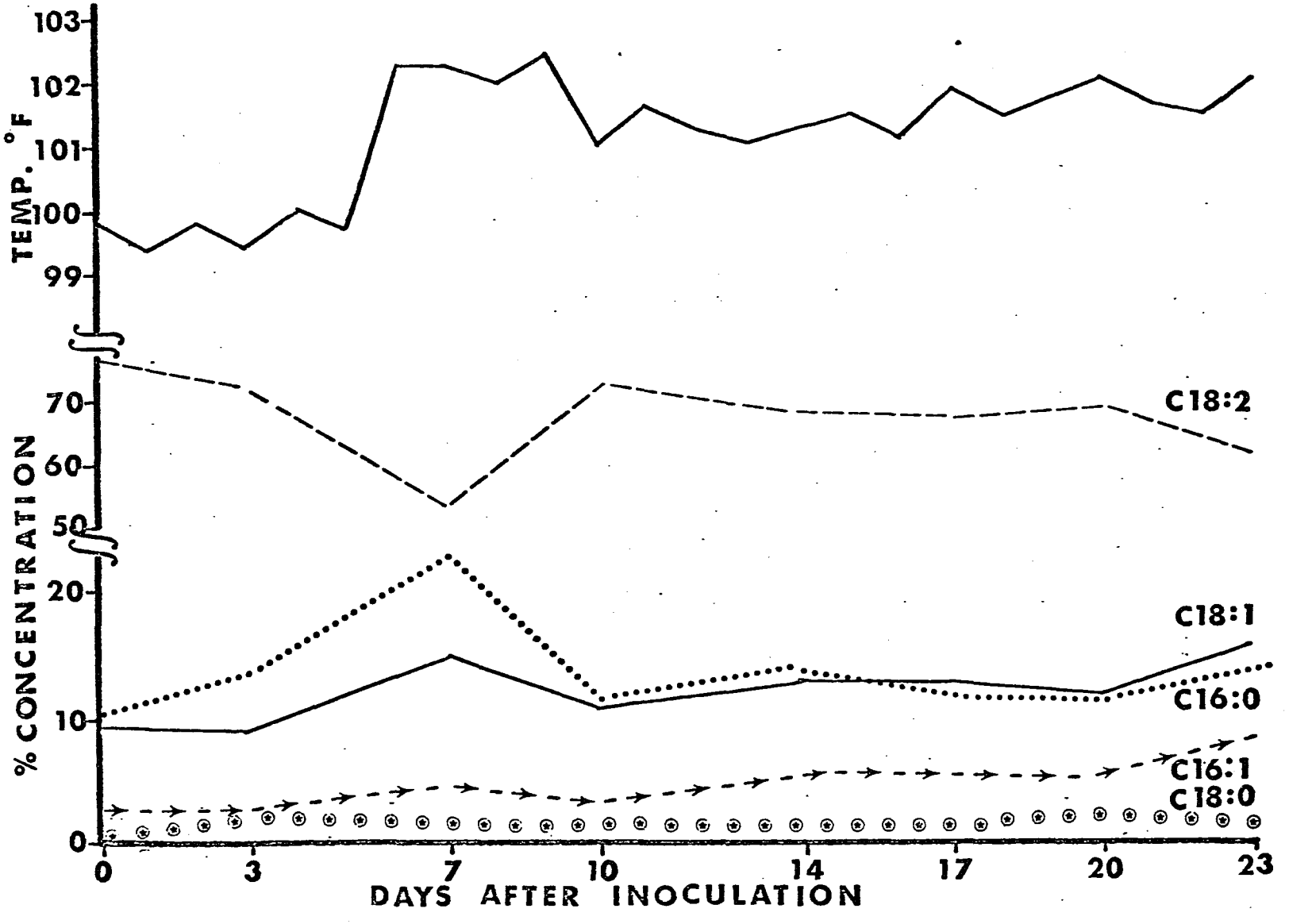
C14:0 each had a tendency to exhibit a 3 peak area similar to that described by Gainer et al. (1966a). C18:0, which peaked on the seventh day after inoculation, declined by the tenth day and remained depressed until the animal died on the twenty-third day. There appeared to be no correlation of fatty acid peaks with the temperature curve (Figure 8). The ratio of the saturated to unsaturated fatty acids in the TG fraction of the plasma appeared to be relatively stable.

The SE fraction of the plasma was primarily composed of C18:2. On the day of infection the concentration of C18:2 was 73.73 percent and on day 7 it decreased to a low of 53.23 percent of the fatty acids (Table 9 and Figure 9). The decrease in the concentration of C18:2 on day 7 was compensated for by increases in C18:1, C16:0, and C16:1 as shown in Figure 9. C18:1, C16:0, and C16:1 remained elevated until death of the horse. The most impressive feature of the fatty acids in the SE fraction of the plasma was the relatively high concentration of unsaturated fatty acids. The ratio varied from 0.13 to 0.22, which was the lowest values for any of the lipid classes.

D. Analyses of the Lipid Classes of the Erythrocytes

The PL fraction of the erythrocytes constitutes about 60 percent of the lipids of mammalian red blood cells (VanDeenen and DeGier, 1964). The PL fraction as seen in this study of horse erythrocytes was composed mainly of C16:0, C18:0, and C18:1. C16:0 decreased from 33.03 percent to 7.25 percent on day 3 after infection. The concentration of

Figure 9. Plots of body temperature and percent of C16:0, C16:1, C18:0, C18:1, and C18:2 of the fatty acids in the sterol ester class of plasma from Horse No. 6.


TEMP. °F
103
102
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% CONCENTRATION
70
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50
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C18:2
C18:1
C16:0
C16:1
C18:0
0
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14
17
20
23
DAYS AFTER INOCULATION

Table 9. Percent of individual fatty acids of the sterol ester fraction from plasma of Horse No. 6.

Fatty Acid	Days After Inoculation							
	0	3	7	10	14	17	20	23
C14:0	0.64	0.52	0.91	0.56	0.61	0.71	0.61	0.61
C16:0	9.20	8.97	14.95	10.42	12.42	12.06	11.72	15.53
C16:1	3.07	2.83	4.41	3.29	5.20	5.42	5.09	8.02
C18:0	0.70	1.96	1.51	1.36	1.28	1.54	1.89	1.17
C18:1	10.11	13.23	22.83	11.31	13.72	11.46	11.22	13.19
C18:2	73.73	70,87	53.23	71.38	64.02	67.45	68.06	61.49
C18:3	0.24	0.23	0.46	0.62	0.63	0.69	0.47	-
C20:0	0.18	0.12	0.09	-	-	-	-	-
C20:1	0.18	0.03	0.18	-	-	0.02	-	-
C21:0	0.08	0.04	0.19	-	0.35	0.10	-	-
C21:1	0.21	0.26	0.04	0.19	0.14	0.11	-	-
C20:4	Tr[a]	-	-	-	-	-	-	-
C22:0	0.35	-	-	-	-	-	-	-
C22:1	-	-	-	-	-	-	-	-
C24:0	0.52	-	-	-	-	-	-	-
S/U[b]	0.13	0.13	0.22	0.14	0.17	0.16	0.16	0.20

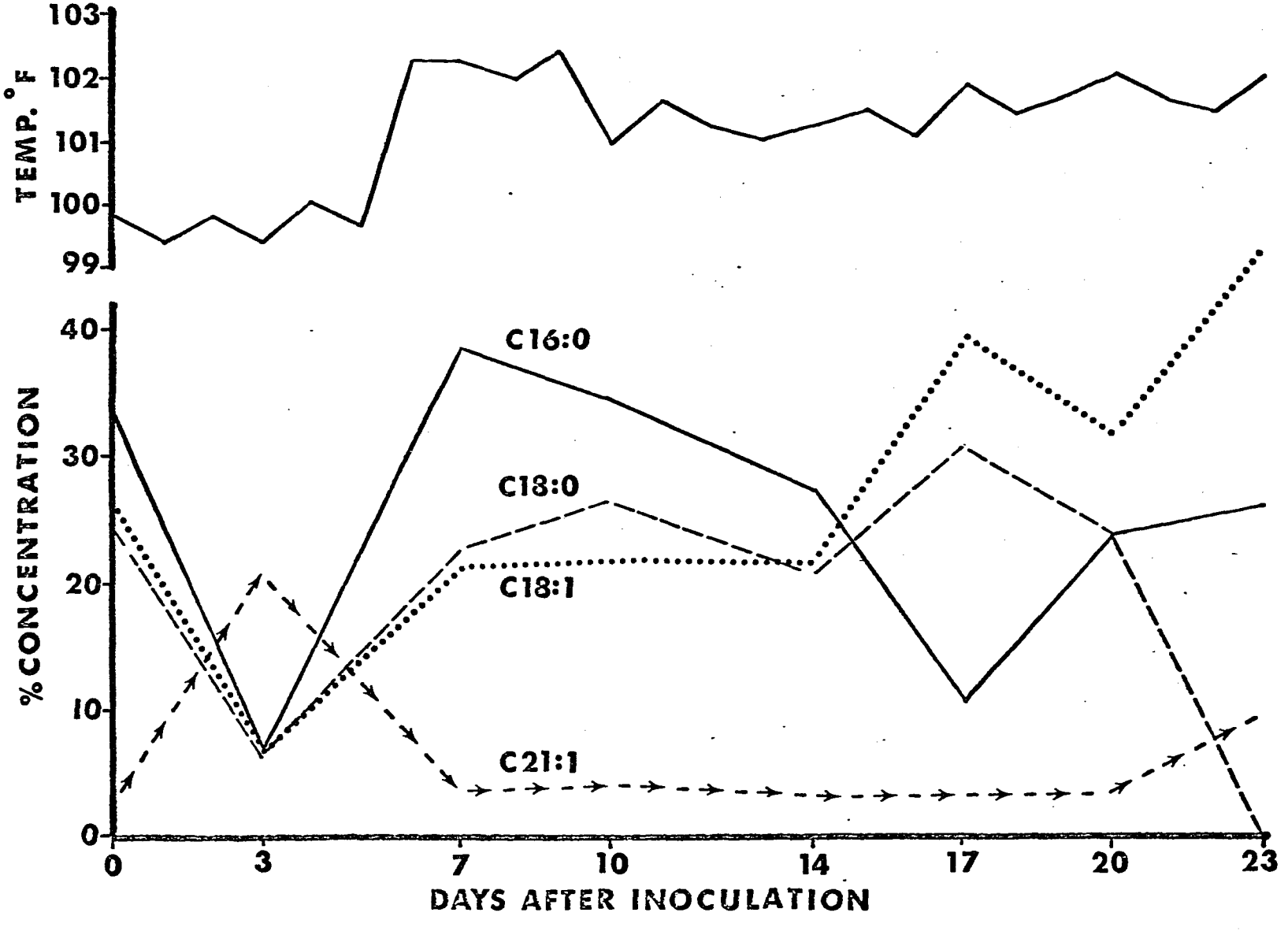
[a]Trace amount.

[b]Saturated to unsaturated fatty acid ratio.

C16:0 increased by day 7 to 38.51 percent. A decrease in C16:0 occurred from day 14 to day 17. C18:0 and C18:1 followed the pattern of C16:0 from day 0 to day 14 (Figure 10). C18:1 increased from day 14 to 39.08 percent and then decreased to 31.81 percent on day 20 only to again increase to 46.84 percent on day 23 (Figure 10 and Table 10). The concentration of C21:1, which usually was less than 5 percent, increased from 2.37 percent on day 0 to 20.91 percent 3 days after infection. However, by day 7 it decreased to less than 5 percent and remained at this approximate level until just before death. The ratio of the saturated to unsaturated fatty acids had an initial value of 1.83 which first decreased and then increased to 2.45 on day 7. The ratio then decreased on day 23 to 0.54. The principle reason for a ratio of 2.45 on day 7 was the increase in the concentrations of C16:0 and the decrease in C21:1. The increase in C18:1 and decrease in C18:0 on day 23 produced the ratio of 0.54 just prior to death.

The FFA fraction of the erythrocytes contained 34.50 percent C16:0 on day 0. There were 2 peaks during the course of experimental EIA. The peaks occurred on day 3 and day 17 when the concentrations were 46.19 percent and 51.02 percent, respectively. C16:0 had decreased to 23.86 percent on day 23 (Figure 11 and Table 11). The concentration of C18:1 remained relatively constant at approximately 17 percent until 14 days after infection. It decreased to 2.04 percent on day 17. C18:1 increased until death on day 23 to 51.09 percent. C18:2 and C18:3 comprised a very low concentration of the FFA and

Figure 10. Plots of body temperature and percent of C16:0, C18:0, C18:1, and C21:1 of the fatty acids in the phospholipid class of erythrocytes from Horse No. 6.


TEMP. °F
103
102
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99
% CONCENTRATION
40
30
20
10
0
C16:0
C18:0
C18:1
C21:1
0
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7
10
14
17
20
23
DAYS AFTER INOCULATION

Table 10. Percent of individual fatty acids of the phospholipid fraction from erythrocytes of Horse No. 6.

Fatty Acid	Days After Inoculation							
	0	3	7	10	14	17	20	23
C14:0	0.46	0.17	0.49	0.33	0.07	0.93	0.52	0.57
C16:0	33.03	7.25	38.51	34.57	27.15	10.50	23.69	25.98
C16:1	0.81	0.33	0.37	0.71	0.44	0.56	1.92	-
C18:0	24.93	6.28	22.86	26.11	20.86	30.57	24.71	0.27
C18:1	26.31	6.56	21.03	21.99	21.25	39.08	31.81	46.84
C18:2	1.15	0.29	0.82	0.80	1.85	2.64	2.11	1.85
C18:3	-	0.38	Tr[a]	0.04	0.16	-	-	0.15
C20:0	0.60	2.96	0.60	0.62	2.25	-	0.28	0.22
C20:1	0.51	1.52	0.60	0.62	0.32	-	-	2.10
C21:0	1.48	6.47	1.11	1.35	3.22	-	1.41	5.89
C21:1	2.37	20.91	3.55	4.12	3.14	3.01	3.45	9.45
C20:4	0.59	5.02	0.99	0.86	1.61	-	-	0.56
C22:0	0.59	3.00	4.01	2.64	0.56	11.10	2.69	-
C22:1	0.19	-	0.60	0.28	2.74	-	-	-
C24:0	1.61	10.42	1.02	2.26	2.01	-	5.88	-
C24:1	0.25	-	-	-	-	-	-	-
C24:U1	2.04	-	-	-	-	-	-	-
S/U[b]	1.83	1.04	2.45	2.30	1.78	1.17	1.50	0.54

[a]Trace amount.

[b]Saturated to unsaturated fatty acid ratio.

Figure 11. Percent of C14:0, C16:0, C18:0, C18:1, and C18:2 of the fatty acids in the free fatty acid class of erythrocytes from Horse No. 6.

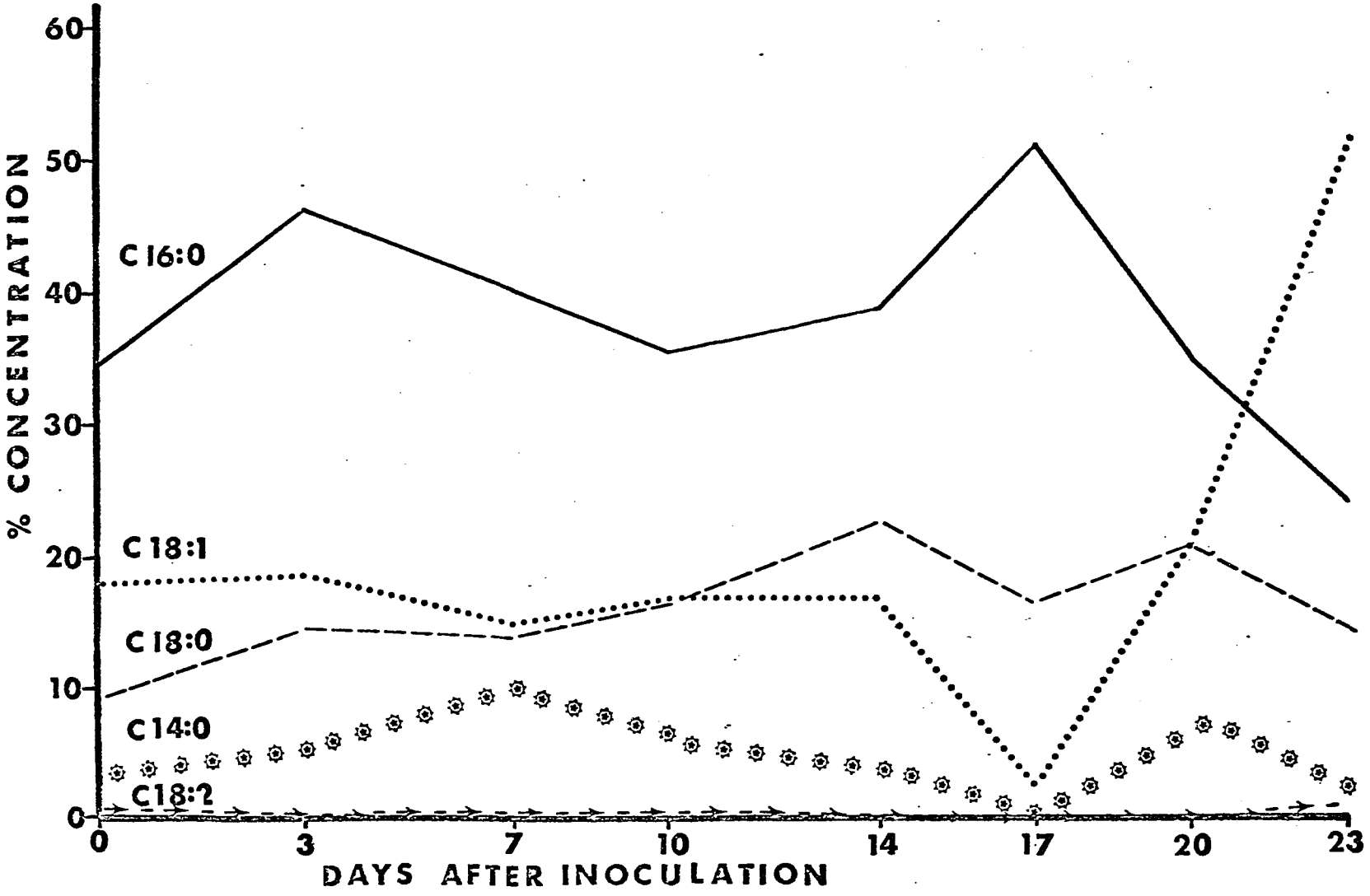
% CONCENTRATION
60
50
40
30
20
10
0
C 16:0
C 18:1
C 18:0
C 14:0
C 18:2
0
3
7
10
14
17
20
23
DAYS AFTER INOCULATION

Table 11. Percent of individual fatty acids of the free fatty acid fraction from erythrocytes of Horse No. 6.

Fatty Acid	Days After Inoculation							
	0	3	7	10	14	17	20	23
C14:0	3.29	5.25	8.96	6.13	3.90	Tr[a]	7.44	2.26
C16:0	34.50	46.19	40.05	35.27	38.57	51.02	34.74	23.86
C16:1	9.55	10.38	10.70	10.40	6.34	10.20	3.22	-
C18:0	9.43	14.44	13.93	16.09	22.67	16.33	20.68	14.30
C18:1	17.76	18.57	14.93	16.31	16.92	2.04	20.98	51.09
C18:2	0.66	-	-	0.19	-	-	Tr	1.19
C18:3	1.68	0.23	-	1.55	0.83	-	-	-
C20:0	1.37	0.98	-	0.50	1.32	-	-	-
C20:1	1.55	0.69	1.24	0.19	0.20	-	-	-
C21:0	0.54	0.52	0.75	-	-	-	0.63	0.40
C21:1	11.49	6.34	4.48	9.07	6.14	20.41	4.02	1.63
C20:4	0.84	0.81	-	0.53	-	-	-	0.17
C22:0	-	1.85	-	-	-	-	-	0.84
S/U[b]	1.12	1.87	2.03	1.51	2.18	2.06	2.24	0.77

[a] Trace amount.

[b] Saturated to unsaturated fatty acid ratio.

never represented more than 1.75 percent of the fatty acids throughout the course of disease. C21:1 had an intial percentage of 11.49, but by the first sampling period on day 3 it had dropped below its initial level (Figure 12). On day 17, C21:1 increased to 20.41 percent only to decrease to below the initial level on day 20 and remain there until the horse died on day 23. Table 11 shows the ratio of saturated to unsaturated fatty acids. On day 23, the unsaturated fatty acids surpassed the saturated fatty acids in concentration. This was primarily due to the relatively large increase in C18:1 and the decrease in C16:0.

The major fatty acids of the TG fractions of the erythrocytes were C16:0 and C18:1 (Table 12). The initial percentages for C16:0 and C18:1 were 30 percent and 21 percent, respectively. Figures 13 and 14 show that C16:0 and C18:1 reacted almost inversely until day 17. From day 17 until 23 days after infection, the changes were almost identical. C18:0 maintained almost constant until day 17 when it increased to about 26 percent; however, it had returned to its initial level by 23 days. The concentrations of C16:1 and C14:0 were almost constant until day 17 when they decreased to less than 2 percent. By day 20 they had nearly returned to their initial levels. C21:1 showed the most drastic change in concentration. On day 0 C21:1 was present only in trace amounts but by day 17 it began to increase. On day 23, C21:1 made up almost 39 percent of the fatty acids of the TG fraction of the erythrocytes (Table 12).

Figure 12. Percent of C16:1, C18:3, and C21:1 of the fatty acids in the free fatty acid class of erythrocytes from Horse No. 6.

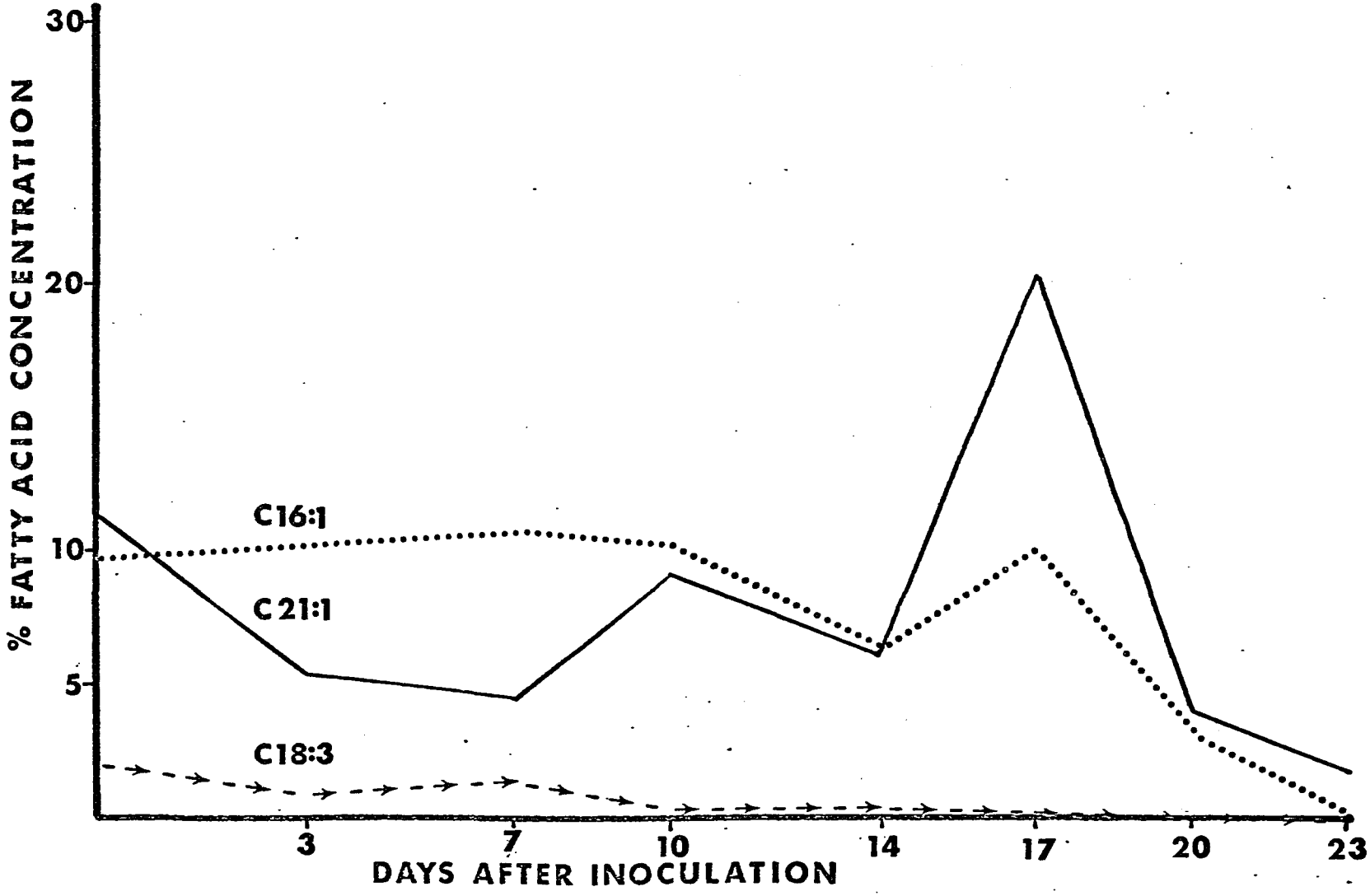
30
20
10
5
% FATTY ACID CONCENTRATION
C16:1
C21:1
C18:3
3
7
10
14
17
20
23
DAYS AFTER INOCULATION

Figure 13. Plots of body temperature and percent of C14:0, C16:0, and C16:1 of the fatty acids in the triglyceride class of erythrocytes from Horse No. 6.

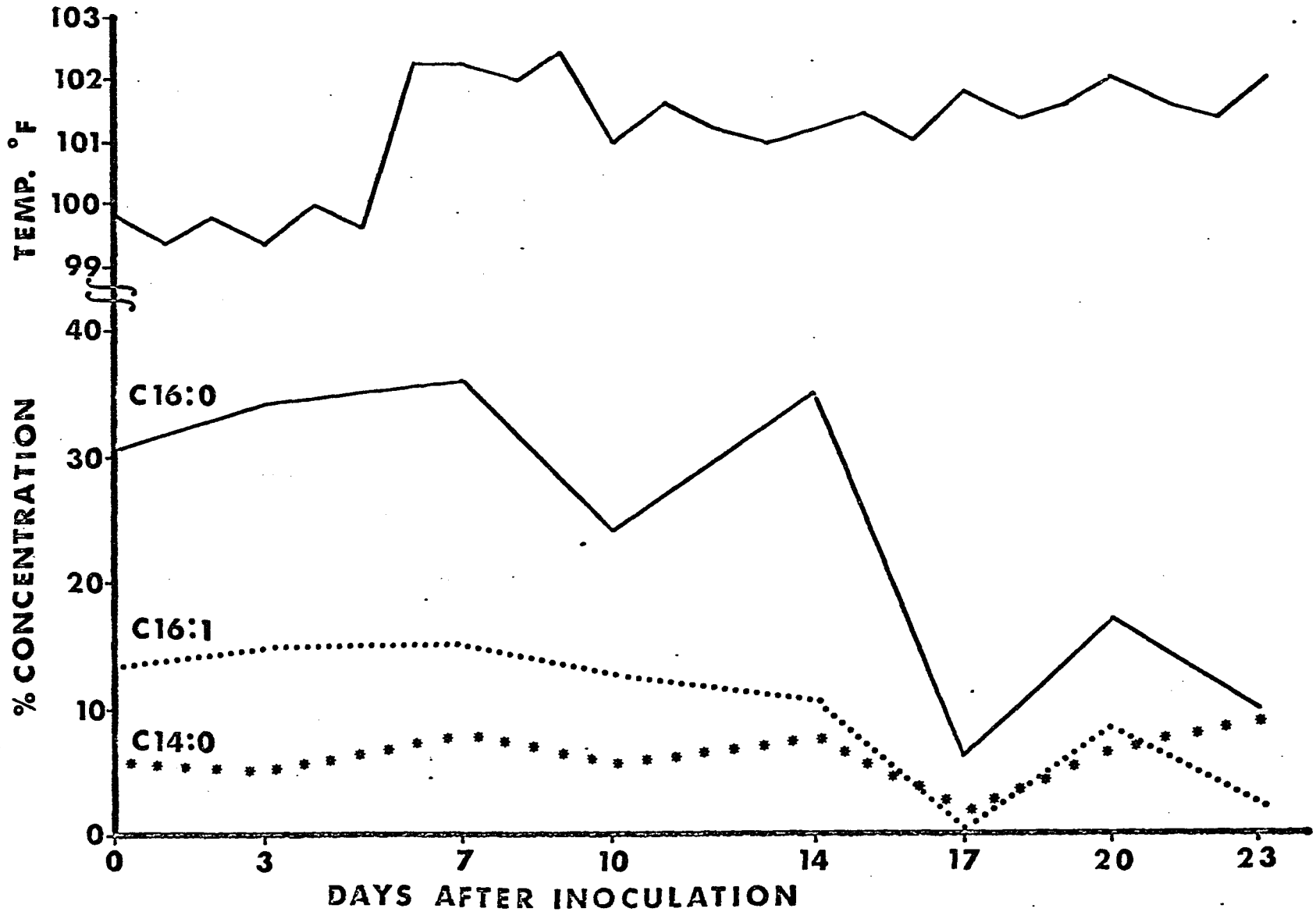
103
102
101
100
99
TEMP. °F
40
30
20
10
0
% CONCENTRATION
C16:0
C16:1
C14:0
0
3
7
10
14
17
20
23
DAYS AFTER INOCULATION

Figure 14. Plots of body temperature and percent of C18:0, C18:1, and C21:1 of the fatty acids in the triglyceride class of erythrocytes of Horse No. 6.

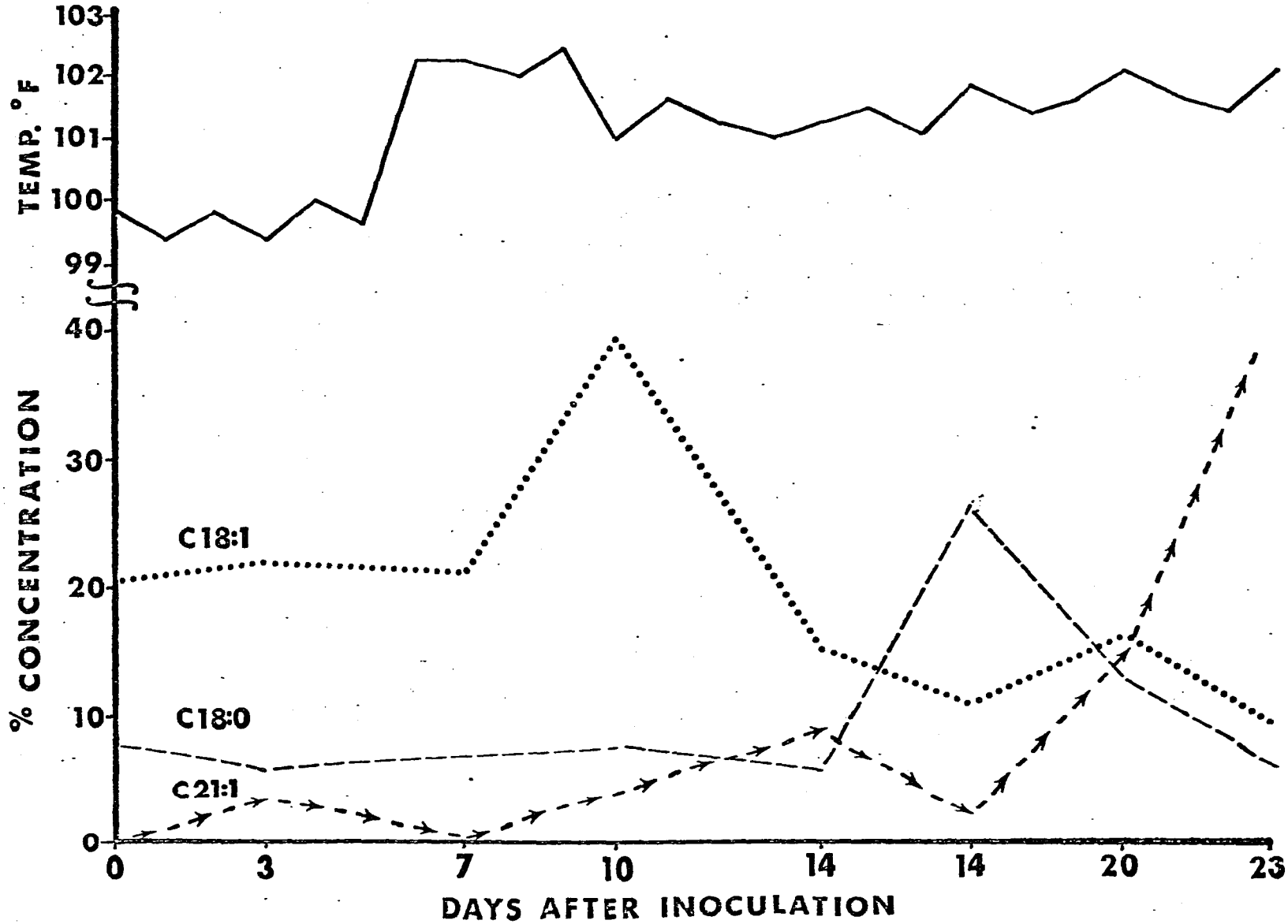
TEMP. °F
103
102
101
100
99
% CONCENTRATION
40
30
20
10
0
C18:1
C18:0
C21:1
0
3
7
10
14
14
20
23
DAYS AFTER INOCULATION

Table 12. Percent of individual fatty acids of the triglyceride fraction from erythrocytes of Horse No. 6.

Fatty Acid	Days After Inoculation							
	0	3	7	10	14	17	20	23
C14:0	5.83	4.95	7.89	5.37	7.17	1.39	6.54	8.91
C16:0	30.69	34.06	36.27	23.94	35.06	5.89	27.04	9.62
C16:1	13.33	14.91	14.98	12.38	10.56	-	8.18	2.29
C18:0	7.64	5.37	6.55	7.60	5.78	26.00	13.09	6.02
C18:1	20.83	22.30	21.29	39.36	15.14	10.57	16.03	9.45
C18:2	1.81	2.19	1.38	0.54	1.20	0.52	2.62	0.93
C18:3	-	1.49	0.84	0.60	2.19	-	0.65	0.93
C20:0	0.42	0.29	0.30	0.33	-	26.00	1.53	-
C20:1	0.83	0.45	0.30	0.27	-	17.85	-	-
C21:0	2.92	-	0.30	-	-	-	-	2.77
C21:1	-	3.67	0.79	3.91	8.57	2.08	14.39	38.46
C20:4	-	-	-	-	-	-	-	1.10
C22:0	-	-	-	-	-	-	-	10.69
C22:1	-	-	-	-	-	-	-	-
S/U[a]	1.29	0.99	1.30	0.65	1.27	1.91	1.15	0.72

[a]Saturated to unsaturated fatty acid ratio.

The SE fraction of the erythrocytes had the most even initial distribution of fatty acids (Figure 15 and 16 and Table 13). The concentrations of C18:0 and C18:1 ranged between 12.82 percent and 14.87 percent on day 0. The concentrations of C16:0 and C18:0 increased on day 3 while C14:0 and C16:1 decreased. C18:0 was not found on day 7 while C16:0 remained elevated to about 25 percent of the SE fraction. During this same period the concentrations of C14:0 and C16:1 increased. All of the major fatty acids of the SE fraction subsequently underwent erratic fluctuations from day 10 until the animal's death at day 23. The saturated fatty acids made up a smaller percentage of the total SE lipid extract than the unsaturated fatty acids only on day 10 and day 23. The S/U of 0.46 on day 23 resulted from the appearance of an unknown C24 and an increase in C21:1 (Table 13).

Additional supporting data of the fatty acid changes of total lipid extract from the plasma and erythrocytes of horses before and after inoculation with the EIA virus are presented in Tables 1 through 3 in the Appendix. Appendix Tables 4 through 7 represent the changes in the fatty acid of the PL, FFA, TG, and SE classes of plasma from Horse No. 4. Tables 8 through 11 represent the fatty acid changes in the PL, FFA, TG, and SE classes of erythrocytes from Horse No. 4. These additional supporting data have not been subjected to rhetoric in this dissertation since the trends of these data are similar.

Figure 15. Plots of body temperature and percent of C14:0, C16:0, and C16:1 of the fatty acids in the sterol ester class of erythrocytes from Horse No. 6.

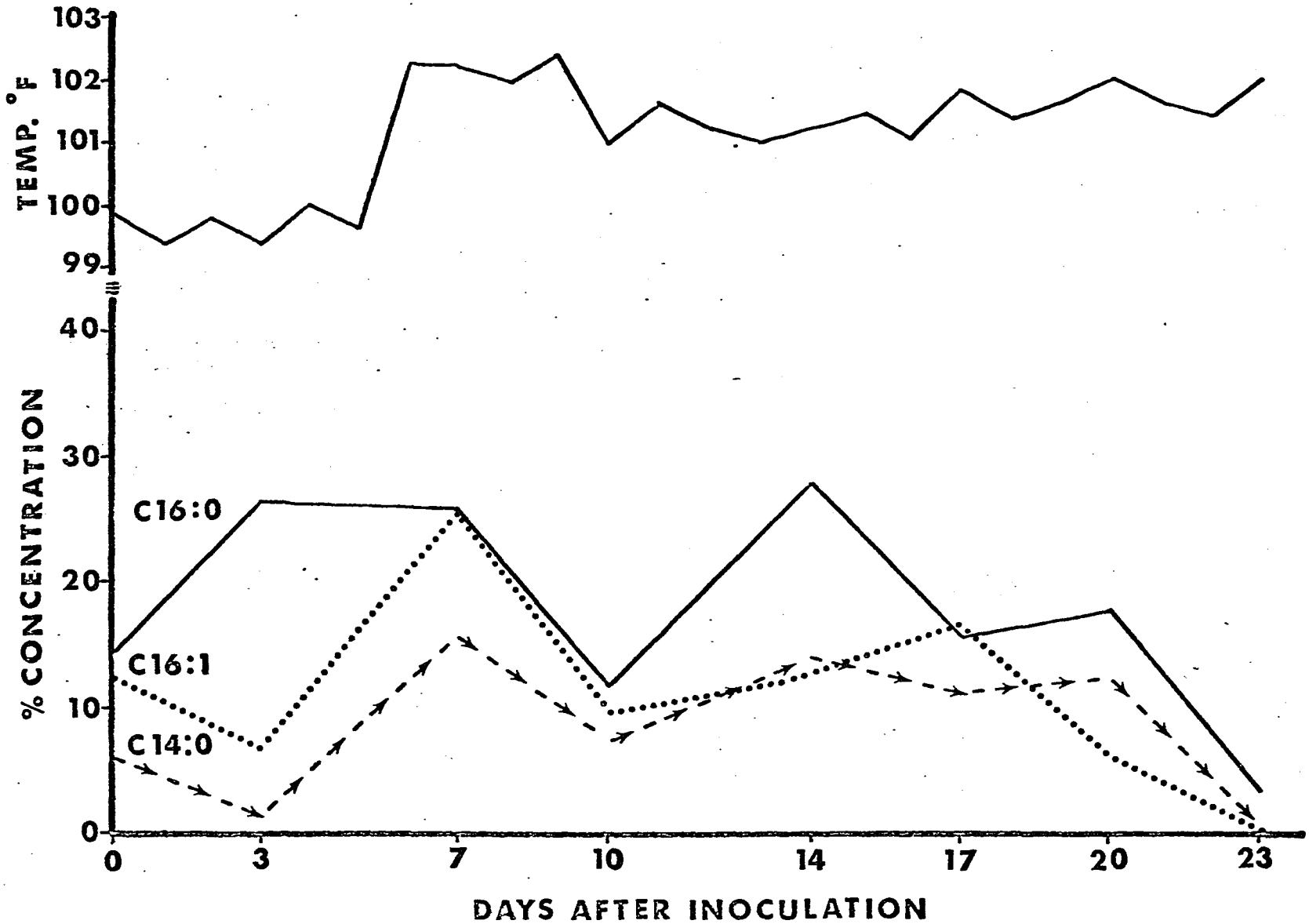
TEMP. °F
103
102
101
100
99
% CONCENTRATION
40
30
20
10
0
C16:0
C16:1
C14:0
0
3
7
10
14
17
20
23
DAYS AFTER INOCULATION

Figure 16. Plots of body temperature and percent of C18:0, C18:1, and C21:1 of the fatty acids in the sterol ester class of erythrocytes from Horse No. 6.

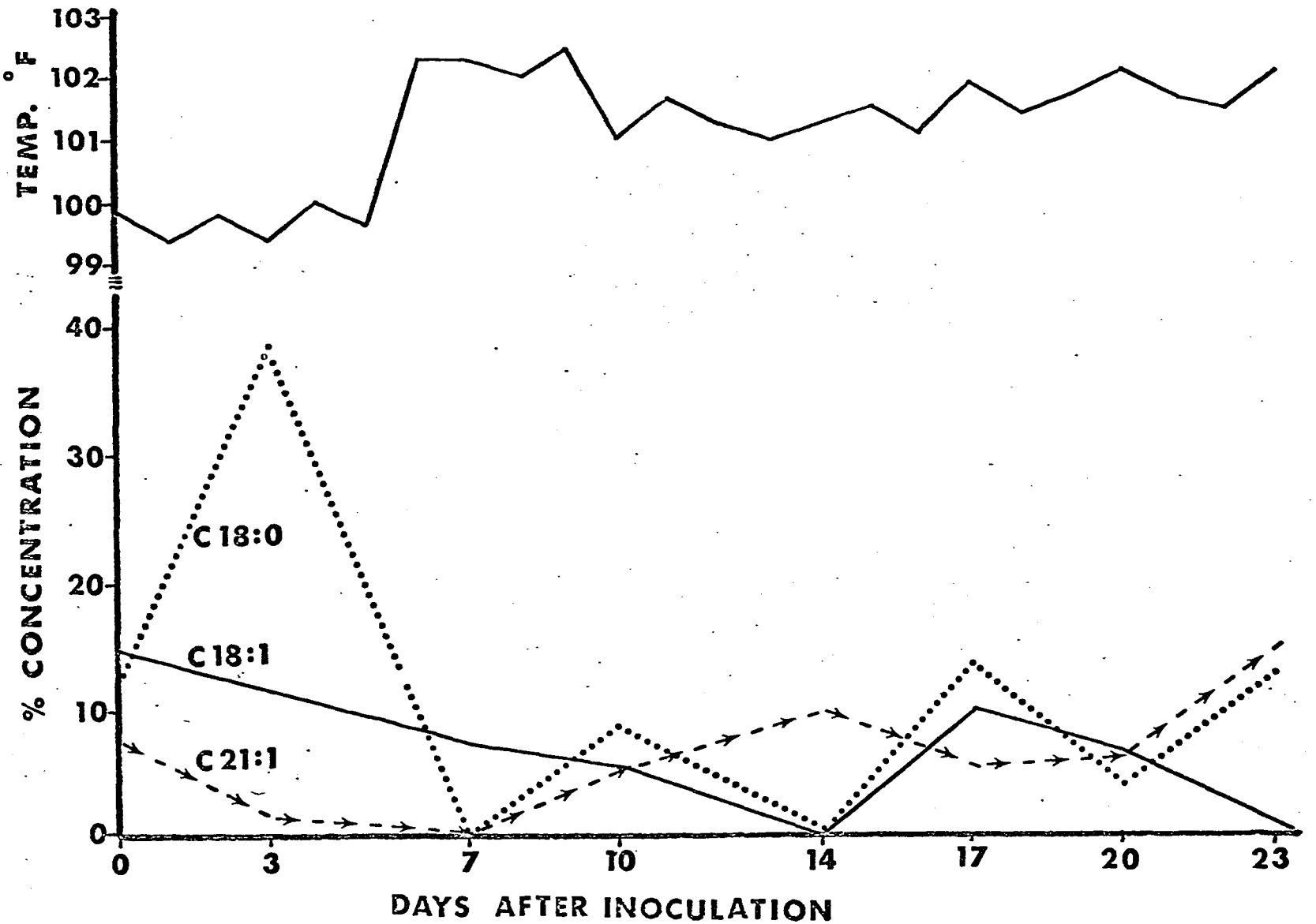
TEMP. °F
103
102
101
100
99
% CONCENTRATION
40
30
20
10
0
C 18:0
C 18:1
C 21:1
0
3
7
10
14
17
20
23
DAYS AFTER INOCULATION

Table 13. Percent of individual fatty acids of the sterol ester fraction from erythrocytes of Horse No. 6.

Fatty Acid	Days After Inoculation							
	0	3	7	10	14	17	20	23
C14:0	6.14	1.88	15.71	7.83	13.95	11.10	12.66	0.91
C16:0	14.32	26.35	25.71	11.86	27.91	15.57	17.93	3.40
C16:1	12.82	6.87	25.71	9.62	12.56	16.84	6.22	0.13
C18:0	12.96	38.68	-	8.95	-	13.21	3.88	12.33
C18:1	14.87	11.95	7.14	5.82	-	9.99	6.33	0.87
C18:2	0.41	-	5.71	5.15	-	-	2.53	-
C18:3	0.68	-	7.14	6.71	-	1.05	-	17.50
C20:0	3.00	5.60	2.86	-	4.19	0.55	-	-
C20:1	1.36	0.19	-	-	1.86	0.44	4.43	0.29
C21:0	1.91	0.52	4.29	4.57	3.72	1.39	11.09	0.14
C21:1	7.09	1.51	-	5.15	9.77	5.27	6.01	19.41
C20:4	-	0.56	-	4.03	2.79	1.33	3.16	18.07
C22:0	-	-	-	2.46	-	4.27	-	0.35
C24:0	-	-	-	-	-	-	-	13.33
C24:U1[b]	-	-	-	-	-	-	-	10.33
S/U[a]	1.02	3.46	1.06	0.97	1.84	1.31	1.58	0.45

[a]Saturated to unsaturated fatty acid ratio.

[b]Unknown saturated and/or branched C24 fatty acids.

E. General Discussion of Data

The majority of the fatty acids in the total lipid extract from the plasma was composed of C18:2, C18:1, C16:0 and C18:0. When TLC was employed to separate the lipid classes, the SE fraction and the PL fraction were found to contain higher percentages of C18:2 than the other fatty acids. Observations made of the TLC plates under ultraviolet light showed that the TG fraction made up an ever-increasing percentage of the total lipid extract from the plasma; the SE fraction steadily decreased in concentration as the disease progressed. The decline in C18:2 in the TG fraction, starting on day 7 (Figure 8) can be correlated to the decline of C18:2 in the total lipid extract from the plasma (Figures 1 and 2). The increase of approximately 10 percent in C18:1 in the total lipid extract from the plasma was found to be due to a slight increase in PL, FFA, TG, and SE fractions of the plasma. Although there was a slight decrease in the percentage of C18:1 in the TG fraction, the marked increase in the total amount of this fraction resulted in the increase shown in C18:1 (Figure 2).

The concentration of C16:0 fluctuated slightly in all lipid classes of the plasma except for the TG fraction. The TG fraction appeared to increase slowly and steadily by approximately 5 percent in C16:0 until just before the animal died (Figure 8). The increased fatty acids in the total lipid extract from plasma fluctuated similarly to the fatty acids in

the PL, FFA, and SE classes. The increase in the percentage of C16:0 in the total lipid extract from plasma can be correlated to the increase of C16:0 in the TG fraction. Analyses showed that there was a gradual increase in the concentration of C16:1 in the total lipid extract and lipid classes during the course of EIA.

In the total lipid extract of erythrocytes, there was a marked decrease in the concentration of C16:0, C18:0, and C18:1 14 days after infection. The concentration returned to normal values by day 17 and remained normal until death. When the lipid classes were analyzed, major decreases in C16:0 occurred in the TG fraction and the PL fraction at day 17. Preinoculation values were attained 20 days after infection in the SE and PL fractions. The concentration of C18:0 drastically decreased from day 20 to day 23.

The decrease in percentage composition in the total lipid extract from the plasma can be correlated to fluctuations in the concentrations of C18:1 of the PL and SE fraction (Tables 1, 6, and 9).

The C24 fatty acids of unknown saturation and/or branching which appeared upon GLC analysis of the total lipid extracts from the erythrocytes were occasionally found in the PL and SE fractions when the fatty acids of the classes were chromatographed.

The data presented in this dissertation represent the changes in the various fatty acids of normal horses and horses which were experimentally infected with EIA from blood of a

known carrier. These percentages should not be considered to be absolute values, as there are individual variations between animals (Tables 1, 3 and 4). The overall trend in fatty acid changes however, should be considered of major importance as individual animals exhibited different percentage ranges of individual fatty acids.

Speculation on the mechanism of the anemia in EIA raises the following hypothesis. The experimentally produced anemia in this study is probably a dilutional type anemia quite similar to that reported by Westerman et al. (1970). The marked increase in the concentration of lipids in blood acts as a diluent for the erythrocytes. In this investigation, horses died within 30 days of inoculation. Perhaps this short time period did not allow time for anemia to develop as a hemolytic anemia. Silver et al. (1964) believed that the hemolytic anemia which accompanied hyperlipemia was due to alterations of the erythrocyte membrane, which resulted in hemolysis. Silver et al. also stated that the degree of hemolysis could be directly correlated to the amount of liver damage as shown on liver function test and liver biopsy.

Westerman et al. (1970) reported that hemolytic anemia developed when the ratio of the plasma to red blood cell cholesterol increased. Data obtained during this investigation showed that the overall levels of fatty acid in the SE fraction were relatively consistent. The SE fraction as observed on TLC plates decreased as the disease progressed. The SE fraction of the plasma proved to be very stable after an initial

fluctuation, while the SE fraction of the erythrocytes exhibited greater fluctuations.

Westerman (1968) postulated that hemolytic anemia of Zieve's syndrome was due to the coating of erythrocytes with lipid. Data from this investigation certainly do not disprove this theory as similar hyperlipemic conditions exist in EIA.

Neerhout (1968) reported that humans with hyperlipemia from liver damage had a marked alteration in the PL fraction of the erythrocytic lipids. The C16:0 and C18:1 increased and the C18:0 decreased in human erythrocytes. Data from our experiments showed marked fluctuations in the fatty acids of the PL fraction and tended to support Neerhout's work. Neerhout stated that the hemolytic anemia was independent of the hyperlipemia and was a result of liver damage.

Calves infected with anaplasmosis experience severe anemias which probably result from surface alterations in the lipids of the circulating erythrocytes (Dimopoullos and Bedell, 1961, 1962, 1965; Dimopoullos, 1964). The data given here lends support to these findings, as there were alterations in the fatty acids of the PL and SE fractions from the erythrocytes. Such alterations or fluctuations in the erythrocytic membrane could also lead to the development of fragile membranes and intravascular hemolysis.

Bartley (1970) reported on the effects of fasting on normal horses. He found an increased lipid content in the blood plasma from the second to the eighteenth day. This increase was primarily due to the TG fraction. He made no

mention of hemolytic anemia associated with this increased plasma lipid. Bartley's findings are identical to findings reported in fasting humans (Zawilaki and Paluszak, 1970). Our experiment supports Bartley's data that the increase in lipid was due to the increase in the TG fraction, which was observed on TLC plates when the lipid classes were separated.

Gainer et al. (1966a) presented data on alterations of blood lipoprotein in horses infected with EIA. They reported that the temperature had a biphasic response. Horses in our experiments did not have a biphasic body temperature curve; however, several of the fatty acids (C16:0, C18:0 and C18:1) from the total lipid extract of the erythrocytes showed a slight triphasic response which was similar to that reported by Gainer et al. Gainer et al. also proposed that the target area for the EIA virus in the horse was probably the hepatic cord cells.

The mode of action for hemolytic anemias have had numerous hypotheses. The list includes: alterations in the erythrocytic membrane (McGuire et al., 1970), coating of erythrocytes with lipid (Westerman et al., 1968), alterations in the enzymatic reactions of the erythrocyte (Kaneko et al., 1969), increased requirements of cellular energy (Kaneko et al., 1969), abnormal lipids lysing erythrocytes (Blass and Dean, 1966), and unidentified effects produced by liver damage (Yamamoto and Koni, 1967).

Data in this dissertation lend support to the fact that alterations occur in the erythrocytic membrane. There

were marked changes in one or more of the fatty acid concentrations in the PL, FFA, TG, and SE fractions of the erythrocytes. The marked lipemia experiences in EIA could certainly present the opportunity for coating the erythrocyte with lipid similar to Westerman's hypothesis that lipid coated erythrocytes have a decreased life span. Abnormal lipids in this study were confined to the long chain fatty acids. C24 fatty acids of unknown saturation and/or branching were present in the PL and SE fraction from the erythrocytes. This study does not prove that these unknown components are responsible for the hemolytic anemia of EIA.

This study lends credence that liver function is altered which results in the marked lipemia seen in EIA. It is possible that liver damage as suggested by Gainer et al. (1966a), and Yamamoto and Kono (1967) could be responsible for the hemolytic anemia of EIA, but additional research will be required to determine this mechanism of the anemia in EIA. Future research should explore the details of liver damage produced in EIA. The altered liver functions and the effect of these altered functions on the erythrocyte may lead to a better understanding of the hemolytic anemia of EIA.

SUMMARY AND CONCLUSIONS

The plasma and erythrocytes of normal horses were subjected to lipid analysis to establish a normal base for the concentrations of fatty acids. These horses were inoculated with blood from a known carrier of EIA virus. Blood samples taken at intervals were divided into plasma and erythrocytes and total lipids were extracted. The total lipid extracts were separated into lipid classes by TLC and the fatty acids analyzed by GLC.

The data obtained showed that the principle fatty acids of the plasma and erythrocytes were C16:0, C18:0, C18:1, C18:2, C18:3, C22:0, and C24:0.

The plasma was composed of a higher percentage of unsaturated fatty acids in all lipid classes while the erythrocytes had a higher percentage of saturated fatty acids. The erythrocytes also had a higher percentage of long chain fatty acids than did the plasma. The erythrocytes showed an increase in the percentage of C24 fatty acids as experimental EIA progressed. The data showed that these compounds were in the PL and SE fractions of the erythrocytes.

The total lipid extract of plasma was primarily composed of C18:2 in the normal horse. The C18:2 decreased as EIA progressed, and C18:1 increased almost directly with

the decreases in the C18:2. These data indicated that the major alterations in the fatty acids of the lipid extracts of the plasma began to take place 7 to 9 days after inoculation. The SE fraction of plasma had a higher percentage of unsaturated fatty acids than the other lipid classes.

The lipid extract from the erythrocytes exhibited extensive fluctuations in major fatty acids of all lipid classes in horses with EIA.

One of the primary purposes of this research was to determine if abnormal lipids were produced which could be responsible for the hemolytic anemia of EIA and possibly serve as a diagnostic aid. These data indicated the presence of an increasing concentration in the series of C24 fatty acids. This research did not prove that these fatty acids were responsible for the hemolytic anemia. This research yielded data which is similar to other studies which indicate that the severe lipemia which developed was due to liver changes.

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APPENDIX

Appendix Table 1. Percent of individual fatty acids of the total lipid extract from plasma of Horse No. 26.

Fatty Acid	Days Before Inoculation					Days After Inoculation								
	19	16	12	8	0	2	9	13	16	20	23	27	34	37
C14:0	0.60	0.69	0.59	0.62	0.62	0.69	0.95	0.58	0.58	0.61	0.57	0.39	1.09	2.22
C16:0	15.78	16.02	16.13	16.59	15.95	17.10	17.80	14.59	8.70	16.56	13.40	17.00	24.37	50.99
C16:1	3.09	2.78	2.03	2.30	1.88	2.33	4.70	3.03	2.86	2.06	1.99	1.72	6.80	4.97
C18:0	19.33	18.06	18.14	17.23	19.00	18.55	7.74	20.06	22.81	19.85	18.21	17.39	12.16	24.34
C18:1	14.73	16.83	15.76	16.77	16.67	15.90	14.62	12.50	13.75	14.35	15.29	11.24	21.22	9.51
C18:2	41.32	42.06	44.15	45.16	41.69	42.80	52.25	46.27	48.34	44.81	46.74	48.15	18.33	5.41
C18:3	0.87	1.36	0.81	0.88	0.77	1.20	0.24	1.56	1.50	1.15	1.46	1.01	8.99	0.51
C20:0	0.65	0.24	0.33	0.18	0.91	0.21	-	0.22	0.19	-	0.37	0.41	1.18	-
C20:1	0.27	0.11	0.29	-	0.41	0.25	-	0.09	0.11	-	0.31	0.21	-	-
S/U[a]	0.60	0.56	0.56	0.53	0.59	0.58	0.37	0.56	0.48	0.59	0.49	0.56	0.57	3.80

[a]Saturated to unsaturated fatty acid ratio.

Appendix Table 2. Percent of individual fatty acids of the total lipid extract from plasma of Horse No. 12.

Fatty Acid	Days Before Inoculation					Days After Inoculation							
	50	48	46	43	41	7	4	0	3	7	10	14	20
C14:0	0.66	0.45	0.99	0.81	0.45	0.79	0.99	0.38	0.89	1.16	2.05	1.33	2.14
C16:0	15.49	11.24	21.36	16.36	12.91	15.58	22.87	15.62	21.83	30.45	13.36	29.49	29.18
C16:1	2.05	1.60	2.22	2.17	2.08	2.64	3.85	0.62	3.56	4.84	4.72	4.48	4.59
C18:0	17.42	13.81	13.59	17.08	22.81	12.47	11.61	14.46	9.80	9.79	15.14	9.30	11.85
C18:1	13.34	10.23	14.83	14.52	11.76	16.21	17.17	10.61	14.70	22.67	12.74	25.13	22.98
C18:2	47.00	56.46	43.01	43.80	43.61	46.75	35.47	55.27	44.43	22.57	37.14	20.70	20.16
C18:3	1.64	1.39	1.46	2.37	1.85	2.55	4.11	1.54	0.22	6.65	2.19	7.44	5.74
C20:0	0.40	2.36	0.15	0.37	0.40	Tr[a]	0.27	0.50	0.33	0.12	1.16	0.21	Tr
C20:1	0.24	0.18	0.19	0.44	0.29	Tr	0.32	Tr	0.22	0.16	Tr	0.26	Tr
C20:2	0.21	0.15	0.17	0.26	0.18	0.26	0.23	0.38	Tr	0.02	Tr	Tr	Tr
S/U[b]	0.52	0.40	0.58	0.54	0.61	0.42	0.58	0.45	0.52	0.73	0.56	0.70	0.81

[a] Trace amount.

[b] Saturated to unsaturated fatty acid ratio.

Appendix Table 3. Percent of individual fatty acids of the total lipid extract from erythrocytes of Horse No. 14.

Fatty Acid	Days Before Inoculation					Days After Inoculation					
	48	11	7	4	0	3	7	10	14	17	20
C14:0	1.00	0.80	Tr[a]	0.89	8.75	1.30	0.55	0.63	0.89	0.99	0.58
C16:0	22.72	26.43	16.50	24.77	24.11	29.50	20.71	23.32	21.51	26.00	23.65
C16:1	3.16	1.97	1.71	2.93	1.27	5.30	2.01	2.57	4.95	3.32	2.92
C18:0	26.61	21.65	38.84	22.00	21.95	23.80	19.34	23.34	19.13	24.05	21.21
C18:1	28.41	25.47	36.29	15.17	19.11	21.60	14.65	23.18	19.81	25.54	22.27
C18:2	4.34	0.76	0.15	0.70	0.11	1.30	1.30	1.42	0.99	1.99	2.32
C18:3	2.58	Tr	1.16	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
C20:0	Tr	2.16	3.36	2.68	2.16	1.80	1.35	1.72	1.10	1.55	1.43
C20:1	Tr	2.48	-	1.90	3.18	0.40	0.20	0.42	0.18	0.45	0.48
C21:1	Tr	2.35	Tr	Tr	2.10	1.30	2.55	3.28	2.44	2.91	1.70
C20:4	Tr	0.50	Tr	Tr	0.32	1.30	1.89	0.05	1.24	1.17	0.53
C22:0	Tr	1.97	1.99	2.60	2.39	1.80	0.99	1.18	6.60	Tr	1.11
C24:0	Tr	9.05	-	16.78	9.03	8.40	4.69	5.12	1.77	4.17	4.14
C24:U1	-	2.03	-	4.82	2.78	2.20	16.88	1.14	Tr	1.30	Tr
C24:U2	-	-	-	-	-	-	10.20	10.12	11.60	5.04	15.54
S/U[b]	1.31	1.75	1.54	2.73	2.37	1.99	0.96	1.31	1.24	1.36	1.14

[a] Trace amount.

[b] Saturated to unsaturated fatty acid ratio.

Appendix Table 4. Percent of individual fatty acids of the phospholipid fraction from plasma of Horse No. 4.

Fatty Acid	Days After Inoculation			
	3	10	14	17
C14:0	0.26	0.15	0.20	0.43
C16:0	5.96	9.07	18.82	14.15
C16:1	0.74	0.92	1.05	1.36
C18:0	35.42	40.44	30.21	26.94
C18:1	9.00	4.43	10.49	11.87
C18:2	44.03	37.86	33.89	32.27
C18:3	0.46	0.41	0.45	1.16
C20:0	0.44	0.37	0.48	0.83
C20:1	0.48	0.33	0.48	0.45
C21:0	0.29	0.31	0.19	0.32
C21:1	0.92	0.98	0.81	-
C20:4	0.07	1.73	0.26	0.74
C22:0		0.20	0.11	0.34
C22:1		0.02	0.85	0.13
S/U[a]	0.76	1.08	1.03	0.89

[a]Saturated to unsaturated fatty acid ratio.

Appendix Table 5. Percent of individual fatty acids of the free fatty acid fraction from plasma of Horse No. 4.

Fatty Acid	Days After Inoculation			
	0	3	10	17
C14:0	2.89	2.49	1.85	1.65
C16:0	22.42	24.96	23.59	28.55
C16:1	-	7.09	10.28	12.13
C18:0	21.72	11.86	6.12	5.18
C18:1	29.39	26.32	37.46	35.00
C18:2	2.84	16.19	10.28	7.73
C18:3	0.24	4.73	8.62	6.60
C20:0	5.21	0.27	0.19	0.07
C20:1	6.40	0.33	0.48	0.25
C21:0	3.78	-	-	0.45
C21:1	4.14	1.57	-	-
C24:0	-	0.42	-	0.20
S/U[a]	1.30	0.71	0.47	0.58

[a]Saturated to unsaturated fatty acid ratio.

Appendix Table 6. Percent of individual fatty acids of the triglyceride fraction from plasma of Horse No. 4.

Fatty Acid	Days After Inoculation 7	10	17
C14:0	2.00	1.78	6.40
C16:0	31.06	32.26	14.48
C16:1	7.11	9.74	12.64
C18:0	8.72	34.50	7.30
C18:1	31.89	8.72	38.61
C18:2	15.39	7.81	9.75
C18:3	4.33	0.18	0.04
C20:0	0.06	0.11	8.65
C20:1	0.28	0.24	0.04
C21:0	0.11	-	0.80
C21:1	0.11	-	-
C20:4	0.28	-	-
C22:0			
C22:1			
S/U[a]	0.71	2.57	0.62

[a] Saturated to unsaturated fatty acid ratio.

Appendix Table 7. Percent of individual fatty acids of the sterol ester fraction from plasma of Horse No. 4.

Fatty Acid	Days After Inoculation					
	0	3	7	10	14	17
C14:0	0.56	0.41	0.39	1.18	2.24	0.47
C16:0	6.21	9.54	8.76	15.71	43.12	12.70
C16:1	1.98	2.95	2.70	5.45	0.17	5.83
C18:0	6.21	1.22	1.34	2.12	35.19	10.15
C18:1	6.21	5.05	11.26	16.23	8.22	68.72
C18:2	5.46	78.64	74.03	52.23	8.76	0.36
C18:3	43.91	0.31	0.17	0.40	0.02	-
C20:0	10.40	0.11	0.26	0.04	0.29	0.13
C20:1	9.34	-	0.29	0.66	-	-
C21:0	-	0.23	-	0.22	0.23	0.15
C21:1	5.59	-	-	-	-	-
C20:4		-	0.26	0.10	0.03	-
C22:0		-	-	-	-	-
C22:1		-	-	-	-	-
S/U[a]	0.32	0.13	0.12	0.25	4.71	0.31

[a]Saturated to unsaturated fatty acid ratio.

Appendix Table 8. Percent of individual fatty acids of the phospholipid fraction from erythrocytes of Horse No. 4.

Fatty Acid	Days After Inoculation					
	0	3	7	10	14	17
C14:0	0.27	-	0.62	0.42	0.29	0.42
C16:0	13.28	36.80	35.09	23.06	17.84	20.53
C16:1	0.59	0.86	1.07	1.85	1.28	2.58
C18:0	11.62	22.44	19.92	20.12	15.05	27.31
C18:1	61.85	26.31	22.02	19.85	17.22	28.91
C18:2	0.34	0.60	1.43	1.35	22.90	3.00
C18:3	0.08	-	0.05	1.84	1.88	0.11
C20:0	0.77	1.38	0.86	4.04	1.23	0.78
C20:1	0.61	0.86	0.74	9.17	3.17	0.53
C21:0	1.58	2.58	2.71	0.17	0.34	1.11
C21:1	2.16	1.72	3.14	0.80	-	3.62
C20:4	0.43	1.38	0.80	1.13	2.23	0.44
C22:0	0.61	-	4.45	4.30	4.18	2.71
C22:1	0.61	-	1.89	0.97	1.36	1.24
C24:0	-	4.13	2.41	-	-	2.91
C24:U1	2.37					1.37
S/U[a]	0.41	2.12	2.12	1.41	0.78	1.37

[a]Saturated to unsaturated fatty acid ratio.

Appendix Table 9. Percent of individual fatty acids of the free fatty acid fraction from erythrocytes of Horse No. 4.

Fatty Acid	Days After Inoculation				
	3	7	10	14	17
C14:0	8.29	5.90	4.62	1.60	39.91
C16:0	36.26	34.99	31.68	13.67	28.82
C16:1	7.82	7.50	1.76	2.20	2.22
C18:0	14.22	15.76	21.34	7.60	6.65
C18:1	18.13	19.16	25.08	8.55	11.09
C18:2	6.04	0.87	1.54	1.29	-
C18:3	-	0.27	0.44	50.66	-
C20:0	-	0.97	0.44	-	-
C20:1	0.59	0.87	0.77	-	-
C21:0	-	0.33	0.22	1.33	0.22
C21:1	2.49	5.16	5.39	-	1.55
C20:4	-	1.10	-	1.41	0.67
C22:0	-	-	-	0.53	-
C22:1	-	-	-	9.53	-
S/U[a]	1.68	1.66	1.67	0.23	4.87

[a] Saturated to unsaturated fatty acid ratio.

Appendix Table 10. Percent of individual fatty acids of the triglyceride fraction from erythrocytes of Horse No. 4.

Fatty Acid	Days After Inoculation					
	0	3	7	10	14	17
C14:0	10.37	5.78	4.88	5.39	9.48	4.71
C16:0	26.85	25.05	28.17	27.75	30.82	27.99
C16:1	7.01	2.00	6.50	12.33	11.03	13.73
C18:0	14.03	12.49	10.83	12.14	13.28	11.31
C18:1	7.09	40.58	29.25	22.16	17.59	23.01
C18:2	3.10	4.51	6.50	4.82	2.50	1.75
C18:3	2.65	0.33	-	1.93	0.26	0.27
C20:0	0.51	0.23	0.49	1.35	0.60	1.21
C20:1	0.16	0.60	0.98	0.96	0.30	0.27
C21:0	-	-	0.60	5.39	0.34	4.44
C21:1	7.01	1.70	1.35	-	0.26	-
C20:4	-	-	0.54	-	0.82	-
S/U[a]	1.99	0.88	1.00	1.23	1.66	1.27

[a]Saturated to unsaturated fatty acid ratio.

Appendix Table 11. Percent of individual fatty acids of the sterol ester fraction from erythrocytes of Horse No.4.

Fatty Acid	Days After Inoculation 0	3	7	10	14
C14:0	3.51	10.90	5.61	5.86	2.93
C16:0	6.59	25.00	11.45	24.92	16.70
C16:1	10.51	9.62	7.88	11.73	16.27
C18:0	2.41	2.88	2.69	2.12	10.09
C18:1	3.18	6.09	4.15	10.67	10.70
C18:2	14.23	12.02	3.37	5.54	2.14
C18:3	2.11	5.45	1.46	3.50	3.12
C20:0	9.92	2.08	1.57	1.79	3.61
C20:1	-	-	1.57	2.85	1.65
C21:0	-	-	-	4.15	2.75
C21:1	-	-	-	2.85	4.40
C20:4	7.86	-	1.01	2.44	1.65
C22:0	4.72				
C24:1					
S/U[a]	0.71	1.23	1.09	0.98	0.90

[a] Saturated to unsaturated fatty acid ratio.

VITA

Donald Gene Luther was born in Elk City, Oklahoma on September 2, 1936. In 1954 he was graduated from Elk City High School. He was honorably discharged from the United States Air Force in 1957. In January 1958 he entered Oklahoma State University and in August married Tama Ruth Wiggins. The father of 3 sons: Euil Eugene Luther born December 30, 1959, Donald Gene Luther, Jr. born February 7, 1965, and Gene Eujohn Luther born September 10, 1969.

Donald Gene Luther received his Bachelor of Science degree in Animal Science in May 1962 and his Doctor of Veterinary Medicine degree in May 1963. He practiced veterinary medicine from 1963 to 1968 in Plaquemine, Louisiana.

In February 1968 he entered the Graduate School of Louisiana State University on a graduate training grant supported by the National Institutes of Health. He is presently a candidate for the Doctor of Philosophy degree in Microbiology, with a minor in Biochemistry.

EXAMINATION AND THESIS REPORT

Candidate: Donald Gene Luther

Major Field: Microbiology

Title of Thesis: Investigation of Blood Lipid Changes in Horses Infected with Equine Infectious Anemia

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

August 30, 1971